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Abstract: In this paper, we present demographic, clinical, anamnestic, cognitive, and functional data, as well as haematological, haematochemical, immunological and genetic parameters of an exceptional individual: Antonino Turturici, a semi-supercentenarian who held the title of the oldest living Italian centenarian from December 28, 2020, to September 23, 2021. The purpose of this study is to offer fresh insights into extreme phenotypes, with a particular focus on immune-inflammatory parameters. To the best of our knowledge, this study represents the first phenotypic investigation of a semi-supercentenarian, illustrating both indicators of age-related immune phenotype (ARIP) recognized as significant gauges of biological ageing and INFLA-score, a metric designed to assess the cumulative impact of inflammatory markers. The aim of this study was, indeed, to advance our understanding of the role of immune-inflammatory responses in achieving extreme longevity. The results bolster the idea that the immune system can play a role in promoting extreme longevity. However, this does not exclude the involvement of other body systems or organs in achieving extreme longevity.

Keywords: ageing; ARIP; biological ageing; inflamm-ageing; INFLA-score; lymphocyte subsets; longevity; semi-supercentenarian

1. Introduction

The ageing of the population is a pressing global concern that is steadily escalating. The increasing ageing of the population poses a growing array of challenges to public health [1]. Healthcare costs in numerous countries have soared due to the rising number of individuals experiencing health issues as they age, leading to a corresponding surge in severe age-related disabilities. Nonetheless, laboratory experiments conducted on models have demonstrated that ageing is not an unalterable process. In fact, interventions aimed at decelerating or delaying the

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ageing process and extending the period of an active life are within reach [2]. Thus, gaining insights into the mechanisms of longevity holds the potential to partially alter the trajectory of the ageing process.

In this context, studying models of healthy ageing and extreme longevity is of paramount importance. Within the group of long-lived individuals (LLIs, >90), centenarians (≥100 years), semi-supercentenarians (≥105 years), and supercentenarians (≥110 years) are subjects of intense investigation. Centenarians, in particular, stand out as the most exemplary individuals in successful ageing. They have managed to evade diseases or endure age-related conditions such as cancer, diabetes, cardiovascular disease, and stroke, making them a shining example of positive biology [3].

However, a note of caution should be taken into account, as the increasing number of centenarians can be attributed to advancements in hygienic and sanitary conditions, as well as improvements in the quality of nutrition. These factors have enabled a greater number of older individuals to achieve exceptional longevity. As a result, the current and even future centenarians are likely to be less rigorously selected compared to those of a few decade ago [4].

Semi-supercentenarians and supercentenarians constitute a highly selected population, comprised of individuals who have survived two World Wars along with a myriad of environmental and microbial challenges, including the Spanish flu [5–7]. Consequently, it is reasonable to deduce that the immune system of these individuals possesses distinct characteristics that empower them to achieve remarkable levels of human longevity. Thus, studying them can shed light on the immune-inflammatory responses that should contribute either positively or negatively to the attainment of extreme longevity [8,9].

In this paper, we present demographic, clinical, anamnestic, cognitive, and functional data, as well as biochemical and genetic parameters of an exceptional individual: Antonino Turturici (A.T.), a semi-supercentenarian who held the title of the oldest living Italian centenarian from December 28, 2020, to September 23, 2021 (Supplementary Figure S1). The purpose of this study is to offer fresh insights into extreme phenotypes, with a particular focus on immune-inflammatory parameters. To best of our knowledge, this is the first phenotypic study of an oldest centenarian showing ageing-related immune phenotype indicators (ARIP), known to be markers of biological age [10], and IFLA-score known to evaluate synergistic effect of inflammatory markers [11].

2. Materials and Methods

Recruitment

The recruitment of Mr. Turturici 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. The Ethic Committee of Palermo University Hospital (Sicily, Italy) approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki and its amendments. Well-trained nutritionists and physicians administered a detailed questionnaire to collect demographic, clinical, and anamnestic data of interest as well as cognitive and functional tests. Our questionnaire includes several sections regarding anamnestic data, cognitive status (mini-mental state examination [MMSE]), activities of daily living (ADL), instrumental activity of daily living (IADL), smoking, alcohol, sleep habits, and health status, such as main pathologies and drugs, geriatric depression scale (GDS), and eating habits [12]. We also investigate the family history. Before the enrolment, his daughter signed the consent form to release the photos and the sensitive data. The semi-supercentenarian underwent venepuncture, 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.

Molecular tests

Genomic DNA was extracted from leukocytes by a commercial kit. We genotyped the single-nucleotide polymorphism (SNP) rs2802292 G-allele (G>T) of Forkhead box O3A (FOXO3A) gene by amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR). Three genotypes

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were analysed: GG, GT, and TT. The size separation was conducted using agarose gel electrophoresis (2%) [12]. We used the EzWayTM Direct ApoE Genotyping Kit (KOMABIOTECH INC) to analyse Apolipoprotein (Apo)E polymorphisms by standard PCR. The genotype was defined by the combination of three alleles ε2, ε3, and ε4. The primer mixture of ApoE genes was enables to perform one-step multiplex PCR. Six genotypes were analysed: ε2/ε2, ε2/ε3, ε3/ε3, ε3/ε4, ε4/ε4, and ε2/ε4. The size separation was conducted using agarose gel electrophoresis (2.5%) [12].

Haematological and haematochemical parameters

Whole blood was used for automated differential leukocyte counts, and the results were expressed as absolute values using the XN-2000 automated haematology analyser from Sysmex. Lymphocyte subsets were identified through flow cytometry analysis conducted using the FACS Canto II (BD) instrument. These tests were carried out respectively at the Department of Laboratory Medicine and the Central Laboratory of Advanced Diagnosis and Biomedical Research at "P. Giaccone" University Hospital in Palermo [9]. Additionally, haematochemical tests, as well as serum electrophoresis, were performed at the Department of Laboratory Medicine, following standard procedures as previously described [12].

Oxidative and inflammatory tests

The evaluation of ox-LDL and Uric Acid (UA) was carried out as previously described [12,13]. C-reactive protein (CRP) values were determined using an immunoturbidimetric assay on the cobas® c 503 analytical unit, while interleukin(IL)-6 values were assessed through an immunoassay test utilizing electrochemiluminescence technology on the cobas® e 801 analytical unit [9]. We also evaluated the Neutrophil/Lymphocyte ratio (NLR) and the Platelet/Lymphocyte ratio (PLR) which have been significantly linked to the occurrence and advancement of various inflammatory conditions [14]. NLR was calculated by dividing the neutrophil count by the lymphocyte count, while PLR was derived by dividing the platelet count by the lymphocyte count. The INFLA-score was calculated for the entire Sicilian population recruited for the DESIGN project (N= 250, age range 19-111 years). The INFLA-score was computed by generating 10-tiles for CRP, leukocyte count, platelet count, and NLR values. These biomarker 10-tiles were assigned scores ranging from lower levels (from -4 to -1) to higher levels (from +4 to +1), with intermediate values receiving a score of 0. Summing the scores of the four components results in the INFLA-score, which ranges between -16 and +16 [11].

Immunological Tests

Serum levels of immunoglobulins A, G, and M (IgA, IgG, IgM) were also measured on Roche Diagnostics cobas® 8000 modular analyser by an immunoturbidimetric assay on cobas® c 503 analytical unit [Ligotti et al, 2023]. For the ageing-related immune phenotype indicators (ARIP), we analysed CD4/CD8 ratio, CD4 and CD8 naïve percentages and the following ratios: TN (naïve)/(TCM (Central Memory)+TEM (Effector Memory)+TEFF (Effector) (referred as TN/TM) in CD4+ and CD8+ T cells [10].

Statistics

No formal statistical analysis has been conducted. For comparison purposes, the reference range values are presented in the various tables, which, unless specified otherwise, pertain to a sample of the Sicilian population aged between 18 and 65 years.

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3. Results

A.T. was born on January 18, 1912, and passed away on September 23, 2021, in Caltabellotta (Agrigento, Sicily, Italy) (Figure 1). He lived his entire life in Caltabellotta. He completed his military service in Sciacca but did not serve on the front lines due to being the only male in his family. His parents, Pellegrino Turturici and Biagia Ragusa, passed away at the ages of 79 (cause unknown) and 93 (due to a heart attack), respectively. They had five other daughters, four of whom lived to advanced ages (101, 94 [died of colon cancer], 92, 91), and one who passed away at the age of 80 due to hepatitis. Antonio was married and had two children, a male who lived for only six days and a female who is currently 62 years old and dealing with hypertension. Antonio himself completed primary school (5 years of education). As a landowner, he had a consistent income throughout his life, which allowed him to live without financial difficulties. He even travelled extensively within Italy and abroad until the age of 88. After his wife passing in 2017, he continued to reside in his own home, receiving care from his daughter.

Figure 1. The picture depicts the semi-supercentenarian, Antonino Turturici, with Dr. Anna Aiello and Prof. Calogero Caruso. Photo used with permission.

He never smoked, slept around 6 hours per night, and drank a few half glasses of wine at table. He was treated with lansoprazole for oesophageal reflux, diuretics and digoxin for heart failure and choline alfoscerate, a dietary supplement. In 2000 he had melena from antiplatelet agents. Over the past few years, he fell multiple times, with the last incident occurring in June 2020 when he fractured his femur. He successfully underwent surgery and was discharged after 4 days without requiring any transfusions.

With a height of 1 meter and 58 centimetres and a weight of 62 kilograms, he had a body mass index (BMI) of 24.8, while the waist circumference measured 85 centimetres. Finally, his blood

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pressure was good (110/60 mmHg), while his heart rate was somewhat elevated (105 beats per minute).

The administration of the GDS demonstrated the absence of depression. Accordingly, he had an optimistic view of his life, which he considered the best imaginable. The MMSE was not administered due to both visual and hearing impairments. The evaluation of activities of daily living (ADL, such as personal hygiene, dressing, bathroom use/continence, walking, eating) showed that assistance has been required (for about 8 years) regarding basic physical needs. Instrumental activities of daily living (IADL, such as, food preparation, financial management, house cleaning, phone use, and medication responsibility) revealed an inability to perform these complex household functioning behaviours for an extended period.

Regarding eating habits during childhood (up to the age of 15), A.T.'s diet was fairly adherent to the Mediterranean diet (MedDiet), due to limited consumption of red meat (once a week) and the consumption of fruits or vegetables twice a day, as well as bread or pasta. Eggs were consumed two to three times a week, while fish and sweets were consumed occasionally. Regarding the consumption of legumes, they were regularly consumed, especially fava beans during the winter and spring.

As for current eating habits, A.T. hasn't shown close adherence to the Mediterranean diet, except for the consumption of grains like pasta, extra virgin olive oil, milk, fruits, and vegetables, which were consumed once a day. Legumes were consumed two to three times a week. On the contrary, there was a high consumption of sweets (such as cookies, small pastries, and sugar) twice a day and of red meat (in pureed form) once a day.-

Table 1 presents the APOE and FOXO3A data. Regarding ApoE, he did not possess either the favourable ε2 allele or the detrimental ε4 allele. Concerning the FOXO3A gene, A.T. did not carry the longevity-associated G SNP [15]. Then, Table 2 illustrates how the haematological values, including the main lymphocyte subsets, fell within the laboratory reference range.

Table 1. Molecular Tests.

Variable	A.T.	Young Adults N = 29	Centenarians N = 22
APOE	N alleles	N alleles	N alleles
ε3	2	5	4
ε2		49	39
ε4		4	1
FOXO3A rs2802292	N alleles	N alleles	N alleles
G		25	17
T	2	33	27

A.T. = Amtonino Turturici; N = Number; Young Adults (18-39 years); Centenarians (100-111 years).

Table 2. Haematological Parameters.

Variable (Unit)	Values	Laboratory Reference Range Value
Red Blood Cells (10^6µL)	4.39	4.20-5.50
Haemoglobin (g/dL)	13.70	12.00-18.00
Platelets (10^3µL)	230	150-450
Leukocytes (10^3µL)	7.61	4.00-11.00
Neutrophils (10^3 µL)	4.12	2.00-8.00
Eosinophils (10^3 µL)	0.37	0.00-0.80
Basophils (10^3 µL)	0.05	0.00-0.20
Monocytes (10^3 µL)	0.94	0.16-1.00
Lymphocytes (10^3 µL)	2.12	1.00-5.00
CD3 (10^3/µL)	1.25	0.81-2.13
CD4 (10^3/µL)	0.70	0.02-1.88
CD8 (10^3/µL)	0.51	0.06-0.74

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Concerning the haematochemical parameters (Table 3), endocrine and iron markers fell within the laboratory reference range. Lipid markers also aligned with the laboratory reference range, although HDL values were at the lower limits. A distinct situation arised with the liver markers, as albumin and total protein values were reduced. On the other hand, transaminases, GGT, and bilirubin values were within the reference range. Concerning the catabolic parameters, creatinine and urea fall within the laboratory reference range, while considering bone markers, ALP values were higher than the reference range, and the Vitamin D values were extremely low.

Table 3. Haematochemical Parameters.

Variable (unit)	Values	Laboratory Reference Range Value
ENDOCRINE MARKERS		
TSH (μIU/mL)	0.56	0.27-4.20
FT3 (pg/mL)	2.65	2.00-4.40
FT4 (ng/dL)	1.31	0.93-1.70
Insulin (μU/mL)	3.28	2.60-24.90
HOMA Index	0.56	0.47-3.19
Glycaemia (mg/dL)	***69***	70-100
LIVER MARKERS		
ALT (U/L)	13	<41
AST (U/L)	23	<40
GGT (U/L)	53	8-61
Bilirubin (mg/dL)	0.85	<1.20
Albumin (g/L)	***33.4***	38-48
Proteins (g/L)	***58.6***	66-87
IRON MARKERS		
Iron (μg/dL)	85	37-145
Ferritin (ng/mL)	91	15-400
Transferrin (mg/dL)	266	200-360
LIPID MARKERS		
Total Cholesterol (mg/dL)	133	<200
LDL (mg/dL)	***64.4***	>65
HDL (mg/dL)	56	>50
Triglycerides (mg/dL)	63	<200
BONE MARKERS		
Osteocalcin ng/mL	33.4	14.00-46.00
ALP (U/L)	***175***	40-129
Calcium (mg/dL)	8.61	8.40-10.20
Magnesium (mg/dL)	2.13	1.60-2.60
Vitamin D (ng/mL)	***3.75***	(>30)
CATABOLIC PARAMETERS		
Creatinine (mg/dL)	1.01	0.5-1.2
Urea (mg/dL)	33.5	16.8-48.5

Values out of range are in bold, italic and underlined. TSH, Thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine; HOMA=homeostasis model assessment; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; LDL, low density lipoprotein; HDL, high density lipoprotein.

As shown in Table 4 (oxidative and inflammatory tests), IL-6 values and INFLA-scores were higher than the control values, whereas the other parameters are within the reference range. In Table 5, serological and cellular immunological tests are presented. In terms of serological tests, IgG and IgM fell within the laboratory reference range, whereas IgA levels were higher. Regarding ARIP, CD4+ Naïve, CD4/CD8 ratio, and TN/TM (CD4) ratio values were within the laboratory reference range, whereas CD8+ Naïve and TN/TM (CD8) ratio were outside the reference range.

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Table 4. Oxidative and inflammatory tests.

Variable (unit)	Values	Laboratory Reference Range Values
LDL Ox (mIU/ml)	47	*44.6-87.3*
Uric Acid (mg/dL)	6.0	2.4-7.0
CRP (mg/dL)	3.37	<5 mg/dL
IL-6 (pg/ml)	**17,8**	<7pg/ml
NLR	1,95	0.92-2.84
PLR	108,02	074.71-193.34
INFLA-score	*8*	-1.25*

Values out of range are in bold, italic and underlined; NLR neutrophil/lymphocyte ratio; PLR Platelet/Lymphocyte ratio; *Mean of the scores of 99 adults (19-65 years).

Table 5. Immunological Tests.

Variable (unit)	Values	Laboratory Reference Range Values
IgG (mg/dL)	1145	700-1600
IgA(mg/dL)	***551***	70-400
IgM (mg/dL)	94.2	40-230
CD4+ Naive (CD45RA+CD27+) (%)	24.0	4-57
CD8+ Naive (CD45RA+CD27+) (%)	***9***	10-78
CD4/CD8	1.37	0.85-5.04
TN/TM (CD4)	0.32	0-05-1.35
TN/TM (CD8)	***0.10***	0.11-3.48

Values out of range are in bold, italic and underlined. TN T naïve; TM T memory.

Finally, Figure 2 illustrates the serum electrophoresis pattern that confirms the immunological and haematological data. In fact, the electrophoretic pattern highlights a decrease in the percentage of albumin, an increase in β2 globulins, and a slight increase the γ globulin.

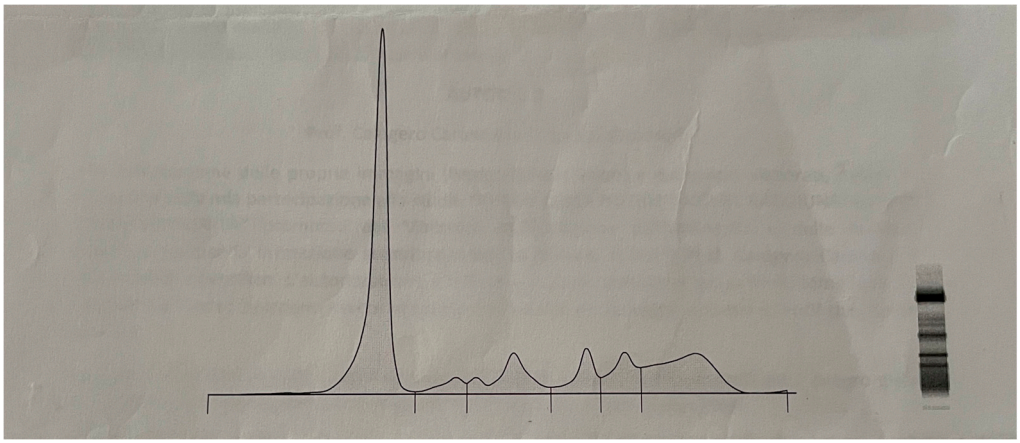

Figure 2. Serum protein electrophoresis percentages (reference range in parentheses). Albumin 51.8% (54.5-65.0), α1 globulins 3.4% (3.1-6.0), α2 globulins 10.8% (7.1-11.8), β1 globulins 7.1 (5.0-7.2), β2globulins, 8.0 (3.2-6.5),γ globulins 18.9 (10.5-18.8).

4. Discussion

It is beyond doubt that A.T. belongs to a long-lived family. However, it is important to clarify that in the human species, family ties do not necessarily imply a genetic connection but can also involve shared environment and lifestyle. As for the genetics, it should not come as a surprise that, in Sicilian centenarians including A.T., there is no association with the FOXO3A and APOE genes. As stated below, it is essential to consider the dynamic interplay between genetic variations and

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environmental factors in the development of individual differences in health [16], and therefore, in longevity. On the other hand, there is emerging evidence that multiple rare and protective variants (varying among different long-lived families) are associated with healthy ageing and extreme longevity [17].

In our Sicilian studies [12,15,18,19], longevity concerns individuals living in small towns or villages situated in the mountainous regions of inland Sicily, far away from major polluted cities. In these villages, the older population has experienced different working conditions compared to those in large cities, as well as distinct lifestyles, including reduced smoking and alcohol abuse and a MedDiet during childhood. In particular, a physically active lifestyle, with regular outdoor activities for commuting to work, is common in these areas due to the generally steep terrain, which is believed to contribute to extended and intense physical activity. This, in turn, improves the cardiorespiratory and immune capacity of the inhabitants [20,21]. Furthermore, in these small towns and villages, people have greater access to family support and social networks. This results in better healthcare and lower mortality rates, especially when there are female offspring. It is intriguing that A.T. spent his entire life in Caltabellotta, a mountainous village (949 meters above sea level) with a population of 3,314, living with his family and receiving care from his daughter. All of this has made his optimistic outlook on life possible, which in turn may have contributed to his longevity [22].

Regarding the diet, it was rich in bioactive Mediterranean foods such as fruits and legumes, but it did not adhere to the traditional MedDiet. Starting from the 1960s, the consumption of meat, fish, fats, and sugars significantly increased in the southern regions of Italy, while the consumption of bread, pasta, cereals, vegetables, and olive oil began to decrease. During this nutritional transition, there was likely a substantial change in A.T.'s diet, as in the rest of Italians. On the other hand, our study of Sicilian centenarians confirms their adherence to the MedDiet at a young age due to food scarcity rather than choice: nutritional options were closely tied to seasons, and the quantity of food was sufficient but never excessive [12,23]. The diet was quite monotonous, often centred around legumes as the primary choice [15,18,19]. All of this could have potentially influenced an individual's ability, including A.T. to achieve extreme longevity through epigenetic modifications [24].

In relation to BMI, usually centenarians are underweight but, in this case, A.T. had normal weight. Moreover, his BMI as well as the waist circumference were within the range. These findings are interesting considering that underweight and overweight conditions, as well as reduction or increase of BMI are unfavourable for longevity [12].

The ApoEε4 allele, known for its pro-inflammatory properties, poses a risk factor for the development of both Alzheimer's disease and cardiovascular diseases. Consequently, it exerts a detrimental influence on longevity, whereas the ε2 allele presence promotes it. On the other hand, the ε3 allele is considered neutral [25]. Our survey on Sicilian centenarians failed to establish a correlation between ε2 and longevity, although the prevalence of the ε4 allele was lower compared to the general population [12]. Accordingly, our semi-supercentenarian carried the genotype ε3/ε3.These findings can be elucidated by a study indicating that the protective impact of ε2 is less pronounced in populations originating from Southern Italy, and that ε4 lacks a harmful effect, hinting at the pivotal role of the MedDiet adhered to by the centenarians during their early years [25].

The transcription factor FOXO3A assumes vital regulatory functions in insulin-like growth factor signalling. Activation of this pathway by a diet abundant in animal proteins and refined sugars curtails its transcription, ultimately fostering unsuccessful ageing [26]. Investigations conducted across several populations have unveiled an association between the SNP rs2802292 (allele G) and longevity, most likely due to escalated FOXO3A expression implicated in homeostatic responses [27]. Conversely, our semi-supercentenarian was of the TT genotype. On the other hand, within the survey encompassing Sicilian centenarians, this longevity-linked association was not evident, plausibly attributed to the aforementioned rationale, this generation early-life diet closely aligned with the MedDiet.

All haematological parameters, including lymphocyte subsets, were within the reference ranges of adult people. Moreover, the values of the lymphocyte subsets were higher or equal to the average values of individuals over ninety years old (data not shown).

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Concerning endocrine markers, the low levels of HOMA index clearly demonstrate good glucose control, reduced risk of type 2 diabetes, and a lower likelihood of associated health complications [28]. A.T. displayed decreased levels of albumin and total proteins, consistent with findings obtained in various centenarian studies [12,29]. Although this data has been used as a biochemical indicator of nutritional status, it is more likely that in the oldest people, including A.T., the low levels reflect the chronic inflammatory state of advanced age, given that albumin is a negative acute-phase protein. All iron markers were found to fall within the laboratory reference range, despite an anticipated decrease in serum iron levels due to the chronic inflammatory state associated with ageing [30].

The lipid marker data we obtained from Sicilian centenarians were largely in agreement with existing literature findings [12]. Literature data indicate that the levels of total cholesterol, LDL, and HDL among centenarians do not significantly differ from or are not lower than those observed in their older adult counterparts, while triglyceride levels were comparable to those of healthy older adults [29]. Nevertheless, the lipid measurements of our semi-supercentenarian virtually fell within the reference range provided by the laboratory.

Interestingly, not only were magnesium values within the expected reference range [12], but also calcium levels were maintained within the range, despite the apparent deficiency in vitamin D, possibly due to inadequate dietary intake or, more likely, limited exposure to sunlight [12,31]. Concerning ALP increase, osteoporosis is often associated with an imbalance between bone resorption and bone formation. Most ALP is produced by osteoblasts. In people with osteoporosis, there can be an alteration in bone turnover, leading to changes in ALP levels. Elevated ALP levels might suggest increased bone remodelling, as the body attempts to restore bone density [32].

It is worth noting that urea and creatinine values remained within the reference range, which contrasts with the anticipated increase associated with the age-related gradual decline in kidney function [33].

UA is the end product of purine metabolism. The role of UA is contentious, as it has been reported to increase oxidative stress, while other studies suggest that UA acts as a scavenger of reactive oxygen species, thus exerting an antioxidant effect [34,35]. Notably, in our recent proteomic study, several proteins exhibited correlations with both age and UA, potentially forming a distinctive signature for healthy ageing [36]. However, UA levels as well as oxLDL levels are within the reference ranges. These findings align with the majority of the literature that reports a decrease in oxidative stress in the long-lived. In fact, in most studies, centenarians have demonstrated lower levels of lipid peroxides and higher plasma levels of the antioxidant vitamin E compared to older controls, suggesting that they might be better equipped to counter oxidative stress [37].

In line with prior studies [21,37–39], we observed a rise in inflammatory markers IL-6 and INFLA-score in our oldest centenarian [9]. IL-6 is known to escalate in response to inflammatory triggers [40]. The INFLA-score contributes to evaluate the possible synergistic effect of inflammatory biomarkers, that may produce multi-collinearity when simultaneously studied, ignoring the variability presented by the differences in units, mean intakes and biological actions [11]. The increase in 2globulins also indicates the chronic inflammatory state of A.T.. In our comprehensive immunological investigation carried out among Sicilians, we noted a progressive rise in inflammatory markers with advancing age, although certain oldest centenarians displayed inflammatory markers akin to those found in younger counterparts [9]. However, this is not the scenario with A.T., whose levels appeared elevated. Nevertheless, the detrimental impacts of inflamm-ageing among centenarians could potentially be mitigated through various mechanisms [41–43]. On an alternate note, considering that A.T. passed away just a year after the blood sample was obtained, these elevated levels align with the notion that inflammation should be a predictive factor for centenarian mortality [15,44].

Regarding the immunological tests, IgG and IgM were within the reference range, while IgA levels are elevated. In fact, IgA values are in perfect alignment with values observed in a previous study [45] involving 11 centenarians (99-108 years old). We did not detect any suspicious band at serum electrophoresis, but second-level investigations for detecting it were not performed, and the

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presence of an inconspicuous monoclonal component cannot be ruled out. Lastly, it is important to highlight that there is emerging evidence suggesting an inflammatory role for IgA [46]. Much more importantly, the semi-supercentenarian displays ARIP marker values such as naive CD4+ cells, the CD4+/CD8+ ratio, and CD4+TN/TM within the range of young adult individuals, suggesting that his immune system has a biological younger age compared to his chronological age [10]. The levels of naive CD8 cells and the TN/TM ratio in CD8 cells below the normal range are known not be associated with biological age or chronological age but with chronic Cytomegalovirus (CMV) infection (A.T., like all ultranonagenarians in Sicily, was CMV-positive) which, even though latent, continually stimulates the immune system [9,21,47].

The potential role of the immune system in contributing to longevity is a subject that lacks universal acceptance. This is due to the fact that centenarians display changes in their immune systems associated with ageing, i.e., immunosenescence [9,47]. Nonetheless, insights garnered from studies involving semi- and supercentenarians, provide support for the notion that the ageing of the immune system in oldest age might be a specialized adaptation rather than a general immune overhaul. From this perspective, it becomes apparent that the oldest centenarians have effectively adjusted to a lifetime of exposure to various antigens. As a result, they have achieved remarkable longevity, a feat that can be partly attributed to modifications in their immune systems [9,47–49]. However, the ARIP of A.T., as briefly discussed earlier, distinctly illustrates that his immune system appears to be "younger" than his chronological age. This finding serves to bolster the idea that the immune system can play a role in promoting longevity.

5. Conclusions

While exploring the role of the immune system in attaining extreme longevity, it is essential to acknowledge that the immune system of older people has been subject to more extensive and in-depth research compared to other bodily systems and organs, primarily due to its amenability to ex vivo studies. On the other hand, immune ageing and the preservation of a relatively robust immune response, may only represent components of the overall deterioration or, conversely, the general well-functioning of the organism, which is regulated by factors beyond the immune system (such as the brain and the endocrine system governed by the brain). Nevertheless, both immune ageing and good immune function could play pivotal roles in the processes of ageing and longevity, respectively. Consequently, the immune system assumes a significantly influential role in the quest for longevity, yet this does not preclude the involvement of other bodily systems or organs.

However, on the whole present findings are consistent with the hypothesis that both semi-supercentenarians and supercentenarians exhibit increasing relative resistance to age-related diseases, approaching the limits of human functional reserve to successfully combat acute causes of death [15,50]..

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title.

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Informed Consent Statement: Written informed consent has been obtained from A.T.'s daughter to publish this paper.

Data Availability Statement: The data that support the findings of this study are availablefrom the corresponding author, upon reasonable request.

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References

1. Christensen, K.; Doblhammer, G.; Rau, R.; Vaupel, J.W. Ageing populations: the challenges ahead. *Lancet*. **2009**, 374(9696), 1196-208. DOI: 10.1016/S0140-6736(09)61460-4.
2. Longo, V.D.; Antebi, A.; Bartke, A.; Barzilai, N.; Brown-Borg, H.M.; Caruso, C.; Curiel, T.J.; de Cabo, R.; Franceschi, C.; Gems, D.; Ingram, D.K.; Johnson, T.E.; Kennedy, B.K.; Kenyon, C.; Klein, S.; Kopchick, J.J.; Lepperdinger, G.; Madeo, F.; Mirisola, M.G.; Mitchell, J.R.; Passarino, G.; Rudolph, K.L.; Sedivy, J.M.; Shadel, G.S.; Sinclair, D.A.; Spindler, S.R.; Suh, Y.; Vijg, J.; Vinciguerra, M.; Fontana, L. Interventions to Slow Aging in Humans: Are We Ready? *Aging Cell*. **2015**, 14(4), 497-510. DOI: 10.1111/acel.12338.
3. Caruso, C.; Passarino, G.; Puca, A.; Scapagnini, G. "Positive biology": the centenarian lesson. *Immun Ageing*. **2012**, 9(1), 5. DOI: 10.1186/1742-4933-9-5.
4. Aiello, A.; Ligotti, M.E.; Cossarizza, A. Centenarian Offspring as a Model of Successful Ageing. In: *Centenarians*; Caruso, C.; Springer, Cham, 2019.
5. Accardi, G.; Aiello, A.; Aprile, S.; Caldarella, R.; Cammarata, G.; Carru, C.; Caruso, C.; Ciaccio, M.; Colomba, P.; Galimberti, D.; Gambino, C.M.; Davinelli, S.; De Vivo, I.; Ligotti, M.E., Vasto, S.; Zinellu, A.; Candore, G. The Phenotypic Characterization of the Cammalleri Sisters, an Example of Exceptional Longevity. *Rejuvenation Res*. **2020**, 23(6), 476-484. DOI: 10.1089/rej.2019.2299.
6. Caruso, C.; Accardi, G.; Aiello, A.; Calabrò, A.; Ligotti, M.E.; Candore, G. Centenarians born before 1919 are resistant to COVID-19. *Aging Clin Exp Res*. **2023**, 35(1), 217-220. DOI: 10.1007/s40520-022-02287-6.
7. Caruso, C.; Marcon, G.; Accardi, G.; Aiello, A.; Calabrò, A.; Ligotti, M.E.; Tettamanti, M.; Franceschi, C.; Candore, G. Role of Sex and Age in Fatal Outcomes of COVID-19: Women and Older Centenarians Are More Resilient. *Int J Mol Sci*. **2023**, 24(3), 2638. DOI: 10.3390/ijms24032638
8. de Castro, M.V.; Silva, M.V.R.; Naslavsky, M.S.; Scliar, M.O.; Nunes, K.; Passos-Bueno, M.R.; Castelli, E.C.; Magawa, J.Y.; Adami, F.L.; Moretti, A.I.S.; de Oliveira, V.L.; Boscardin, S.B.; Cunha-Neto, E.; Kalil, J.; Jouanguy, E.; Bastard, P.; Casanova, J.L.; Quiñones-Vega, M.; Sosa-Acosta, P.; Guedes, J.S.; de Almeida, N.P.; Nogueira, F.C.S.; Domont, G.B.; Santos, K.S.; Zatz, M. The oldest unvaccinated Covid-19 survivors in South America. *Immun Ageing*. **2022**, 19(1), 57. DOI: 10.1186/s12979-022-00310-y. Erratum in: *Immun Ageing*. **2022**, 19(1):61.
9. Ligotti, M.E.; Accardi, G.; Aiello, A.; Aprile, S.; Calabrò, A.; Caldarella, R.; Caruso, C.; Ciaccio, M.; Corsale, A.M.; Dieli, F.; Di Simone, M.; Giammanco, G.M.; Mascarella, C.; Akbar, A.N.; Meraviglia, S.; Candore, G. Sicilian semi- and supercentenarians: Identification of age-related T cell immunophenotype to define longevity trait. *Clin. Exp. Immunol*. **2023**. Epub ahead of print.
10. Ramasubramanian, R.; Meier, H.C.S.; Vivek, S.; Klopack, E.; Crimmins, E.M.; Faul, J.; Nikolich-Žugich, J.; Thyagarajan, B. Evaluation of T-cell aging-related immune phenotypes in the context of biological aging and multimorbidity in the Health and Retirement Study. *Immun Ageing*. **2022**, 19(1), 33. DOI: 10.1186/s12979-022-00290-z.
11. Pounis, G.; Bonaccio, M.; Di Castelnuovo, A.; Costanzo, S.; de Curtis, A.; Persichillo, M.; Sieri, S.; Donati, M.B.; Cerletti, C.; de Gaetano, G., Iacoviello, L. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. *Thromb Haemost*. **2016**, 115(2), 344-52. DOI: 10.1160/TH15-06-0487.
12. Aiello, A.; Accardi, G.; Aprile, S.; Caldarella, R.; Carru, C.; Ciaccio, M.; De Vivo, I.; Gambino, C.M.; Ligotti, M.E.; Vasto, S.; Zinellu, A.; Caruso, C.; Bono, F.; Candore, G. Age and Gender-related Variations of Molecular and Phenotypic Parameters in A Cohort of Sicilian Population: from Young to Centenarians. *Aging Dis*. **2021**, 12(7), 1773-1793. DOI: 10.14336/AD.2021.0226.
13. Aiello, A.; Accardi, G.; Aprile, S.; Caldarella, R.; Cammarata, G.; Carru, C.; Caruso, C.; Ciaccio, M.; De Vivo, I.; Gambino, C.M.; Ligotti, M.E.; Vasto, S.; Candore, G. Pro-inflammatory status is not a limit for longevity: case report of a Sicilian centenarian. *Aging Clin Exp Res*. **2021**, 33(5), 1403-1407. DOI: 10.1007/s40520-020-01628-7.
14. Zinellu, A., Mangoni, A.A. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio and disease activity in rheumatoid arthritis: A systematic review and meta-analysis. *Eur J Clin Invest*. **2023**, 53(2), e13877. DOI: 10.1111/eci.13877

15. Accardi, G.; Aprile, S.; Candore, G.; Caruso, C.; Cusimano, R.; Cristaldi, L.; Di Bona, D.; Duro, G.; Galimberti, D.; Gambino, C.M.; Ligotti, M.E.; Mazzucco, W.; Vasto, S.; Aiello, A. Genotypic and Phenotypic Aspects of Longevity: Results from a Sicilian Survey and Implication for the Prevention and Treatment of Age-related Diseases. *Curr Pharm Des*. **2019**, 25, 228-235. DOI: 10.2174/1381612825666190313115233.
16. Boyce, W.T.; Sokolowski, M.B.; Robinson, G.E. Genes and environments, development and time. *Proc Natl Acad Sci U S A*. **2020**, 117(38), 23235-23241. DOI: 10.1073/pnas.2016710117.
17. Caruso, C.; Ligotti, M.E.; Accardi, G.; Aiello, A.; Duro, G.; Galimberti, D.; Candore, G. How Important Are Genes to Achieve Longevity? *Int J Mol Sci*. **2022**, 23(10), 5635. DOI: 10.3390/ijms23105635.
18. Vasto, S.; Rizzo, C.; Caruso, C. Centenarians and diet: What they eat in the Western part of Sicily. *Immun Ageing*. **2012**, 9, 1.
19. Vasto, S.; Scapagnini, G.; Rizzo, C.; Monastero, R.; Marchese, A.; Caruso, C. Mediterranean diet and longevity in Sicily: survey in a Sicani Mountains population. *Rejuvenation Res*. **2012**, 15(2), 184-8. DOI: 10.1089/rej.2011.1280.
20. Poulain, M.; Pes, G.M.; Grasland, C.; Carru, C.; Ferrucci, L.; Baggio, G.; Franceschi, C.; Deiana, L. Identification of a geographic area characterized by extreme longevity in the Sardinia island: the AKEA study. *Exp Gerontol*. **2004**, 39(9), 1423-9. DOI: 10.1016/j.exger.2004.06.016.
21. Caruso, C.; Ligotti, M.E.; Accardi, G.; Aiello, A.; Candore, G. An immunologist's guide to immunosenescence and its treatment. *Expert Rev Clin Immunol*. **2022**, 18(9), 961-981. DOI: 10.1080/1744666X.2022.2106217.
22. Rea, J.N.M.; Broczek, K.M.; Cevenini, E.; Celani, L.; Rea, S.A.J.; Sikora, E.; Franceschi, C.; Fortunati, V.; Rea, I.M. Insights Into Sibling Relationships and Longevity From Genetics of Healthy Ageing Nonagenarians: The Importance of Optimisation, Resilience and Social Networks. *Front Psychol*. **2022**, 13, 722286. DOI: 10.3389/fpsyg.2022.722286.
23. Vasto, S.; Buscemi, S.; Barera, A.; Di Carlo, M.; Accardi, G.; Caruso, C. Mediterranean diet and healthy ageing: A Sicilian perspective. *Gerontology* **2014**, 60, 508–518.
24. Puca, A.A.; Spinelli, C.; Accardi, G.; Villa, F.; Caruso, C. Centenarians as a model to discover genetic and epigenetic signatures of healthy ageing. *Mech Ageing Dev.* **2018**, 174:95–102.
25. Gurinovich, A.; Andersen, S.L.; Puca, A.; Atzmon, G.; Barzilai, N.; Sebastiani, P. Varying Effects of APOE Alleles on Extreme Longevity in European Ethnicities. *J Gerontol A Biol Sci Med Sci*. **2019**, 74(Suppl_1), S45-S51. DOI: 10.1093/gerona/glz179.
26. Aiello, A.; Accardi, G.; Candore, G.; Gambino, C.M.; Mirisola, M.; Taormina, G.; Virruso, C.; Caruso, C. Nutrient sensing pathways as therapeutic targets for healthy ageing. *Expert Opin Ther Targets*. **2017**, 21(4), 371-380. DOI: 10.1080/14728222.2017.1294684.
27. Caruso, C.; Aiello, A.; Accardi, G.; Ciaglia, E.; Cattaneo, M.; Puca, A. Genetic Signatures of Centenarians: Implications for Achieving Successful Aging. *Curr Pharm Des*. **2019**, 25(39), 4133-4138. DOI: 10.2174/1381612825666191112094544.
28. Horáková, D.; Štěpánek, L.; Janout, V.; Janoutová, J.; Pastucha, D.; Kollárová, H.; Petráková, A.; Štěpánek, L.; Husár, R.; Martiník, K. Optimal Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Cut-Offs: A Cross-Sectional Study in the Czech Population. *Medicina (Kaunas)*. **2019**, 55(5), 158. DOI: 10.3390/medicina55050158
29. Hausman, D.B.; Fischer, J.G.; Johnson, M.A. Protein, lipid, and hematological biomarkers in centenarians: definitions, interpretation and relationships with health. *Maturitas*. **2012**, 71(3), 205-12. DOI: 10.1016/j.maturitas.2011.12.002.
30. Wawer, A.A.; Jennings, A.; Fairweather-Tait, S.J. Iron status in the elderly: A review of recent evidence. *Mech Ageing Dev*. **2018**, 175, 55-73. DOI: 10.1016/j.mad.2018.07.003.
31. Passeri, G.; Vescovini, R.; Sansoni, P.; Galli, C.; Franceschi, C.; Passeri, M.; Italian Multicentric Study on Centenarians (IMUSCE). Calcium metabolism and vitamin D in the extreme longevity. *Exp Gerontol.* **2008**, 43(2), 79-87. DOI: 10.1016/j.exger.2007.06.013.
32. Kuo, T.R.; Chen, C.H. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark Res*. **2017**, 5, 18. DOI: 10.1186/s40364-017-0097-4.
33. Lio, D.; Malaguarnera, M.; Maugeri, D.; Ferlito, L.; Bennati, E.; Scola, L.; Motta, M.; Caruso, C. Laboratory parameters in centenarians of Italian ancestry. *Exp Gerontol*. **2008**, 43(2), 119-22. DOI: 10.1016/j.exger.2007.06.005.
34. Yu, M.A.; Sánchez-Lozada, L.G.; Johnson, R.J.; Kang, D.H. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. *J Hypertens*. **2010**, 28(6), 1234-42.
35. El Ridi, R.; Tallima, H. Physiological functions and pathogenic potential of uric acid: A review. *J Adv Res*. **2017**, 8(5), 487-493. DOI: 10.1016/j.jare.2017.03.003.
36. Siino, V.; Ali, A.; Accardi, G.; Aiello, A.; Ligotti, M.E.; Mosquim, S.J.; Candore, G.; Caruso, C.; Levander, F.; Vasto, S. Plasma proteome profiling of healthy individuals across the life span in a Sicilian cohort with long-lived individuals. *Aging Cell*. **2022**;21(9):e13684. doi: 10.1111/acel.13684.

37. Accardi, G.; Ligotti, M.E.; Candore G. Phenotypic Aspects of Longevity. In *Centenarians*; Caruso, C.; Springer, Cham, 2019.
38. Franceschi, C.; Bonafè, M.; Valensin, S.; Olivieri, F.; De Luca, M.; Ottaviani, E.; De Benedictis, G. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. **2000**, 908:244-54. DOI: 10.1111/j.1749-6632.2000.tb06651.x.
39. Caruso, C.; Accardi, G.; Aiello, A.; Calabrò, A.; Ligotti, M.E.; Candore, G. Lessons from Sicilian Centenarians for Anti-Ageing Medicine. The Oxi-Inflammatory Status. *Transl Med UniSa*. **2022**, 24(2), 16-23. DOI: 10.37825/2239-9754.1036.
40. Mantovani, A.; Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. N Engl J Med. **2023**, 388(5), 439-452. DOI: 10.1056/NEJMra2206346.
41. Accardi, G.; Bono, F.; Cammarata, G.; Aiello, A.; Herrero, M.T.; Alessandro, R.; Augello, G.; Carru, C.; Colomba, P.; Costa, M.A.; De Vivo, I.; Ligotti, M.E.; Lo Curto, A.; Passantino, R.; Taverna, S.; Zizzo, C.; Duro, G.; Caruso, C.; Candore, G. miR-126-3p and miR-21-5p as hallmarks of bio-positive ageing; correlation analysis and machine learning prediction in young to ultra-centenarian Sicilian population. *Cells*. **2022**, 11, 1505.
42. Pinti, M.; Gibellini, L.; Lo Tartaro, D.; De Biasi, S.; Nasi, M.; Borella, R.; Fidanza, L.; Neroni, A.; Troiano, L.; Franceschi, C.; Cossarizza, A. A Comprehensive Analysis of Cytokine Network in Centenarians. *Int J Mol Sci*. **2023** ;24(3):2719. doi: 10.3390/ijms24032719.
43. Zhou, L.; Ge, M.; Zhang, Y.; Wu, X.; Leng, M.; Gan, C.; Mou, Y.; Zhou, J.; Valencia, C.A., Hao, Q.; Zhu, B.; Dong, B.; Dong, B. Centenarians alleviate inflammaging by changing the ratio and secretory phenotypes of T helper 17 and regulatory T cells. *Front Pharmacol*. **2022**, 13, 877709.
44. Arai, Y.; Martin-Ruiz, C.M.; Takayama, M.; Abe, Y.; Takebayashi, T.; Koyasu, S.; Suematsu, M.; Hirose, N.; von Zglinicki, T. Inflammation, but not telomere length, predicts successful ageing at extreme old age: A longitudinal study of semi-supercentenarians. *EBioMedicine* **2015**, 2, 1549-1558.
45. Listì, F.; Candore, G.; Modica, M.A.; Russo, M.; Di Lorenzo, G.; Esposito-Pellitteri, M.; Colonna-Romano, G.; Aquino, A.; Bulati, M.; Lio, D.; Franceschi, C.; Caruso, C. A study of serum immunoglobulin levels in elderly persons that provides new insights into B cell immunosenescence. *Ann N Y Acad Sci*. **2006**, 1089, 487-95. DOI: 10.1196/annals.1386.013.
46. Hansen, I.S.; Baeten, D.L.P.; den Dunnen, J. The inflammatory function of human IgA. *Cell Mol Life Sci*., 76(6), 1041-1055. DOI: 10.1007/s00018-018-2976-8.
47. Ligotti, M.E.; Aiello, A.; Accardi, G.; Aprile, S.; Bonura, F.; Bulati, M.; Gervasi, F.; Giammanco, G.M.; Pojero, F.; Zareian, N.; Caruso, C.; Farzaneh, F.; Candore, G. Analysis of T and NK cell subsets in the Sicilian population from young to supercentenarian: The role of age and gender. *Clin. Exp. Immunol*. **2021**, 205, 198–212.
48. Hashimoto, K.; Kouno, T.; Ikawa, T.; Hayatsu, N.; Miyajima, Y.; Yabukami, H.; Terooatea, T.; Sasaki, T.; Suzuki, T.; Valentine, M.; et al. Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. *Proc. Natl. Acad Sci. USA* **2019**, 116, 24242–24251.
49. Karagiannis, T.T.; Dowrey, T.W.; Villacorta-Martin, C.; Montano, M.; Reed, E.; Belkina, A.C.; Andersen, S.L.; Perls, T.T.; Monti, S.; Murphy, G.J.; et al. Multi-modal profiling of peripheral blood cells across the human lifespan reveals distinct immune cell signatures of aging and longevity. *EBioMedicine* **2023**, 90, 104514.
50. Andersen, S.L.; Sebastiani, P.; Dworkis, D.A.; Feldman, L.; Perls, T.T. Health span approximates life span among many supercentenarians: compression of morbidity at the approximate limit of life span. *J Gerontol A Biol Sci Med Sci*. **2012**, 67(4), 395-405. DOI: 10.1093/gerona/glr223.