



UNIVERSITÀ DEL PIEMONTE ORIENTALE

Doctorate course in Food, Health and Longevity

XVII cycle

Doctoral dissertation

**“Exploring Socioeconomic Determinants of Health: Insights from an Italian Ageing
Study and Breast Cancer Screening Disparities in Canada”**

Supervisor:

Chiar.mo Prof. Fabrizio Faggiano

Co-supervisor

Prof. Saverio Stranges

PhD Candidate:

Chiara Aleni

Academic year: 2023/2024

DICHIARAZIONE E AUTORIZZAZIONE AL RILEVAMENTO ANTIPLAGIO

(Art. 47 e Art. 38 del D.P.R. 28 dicembre 2000, n. 445) e s.m.i.

esente da bollo ai sensi dell'art. 37 D.P.R. 445/2000 e s.m.i.

Il/La sottoscritto/a Chiara Aleni nato/a a Milano
prov (MI) il 8/2/96, regolarmente iscritto/a per l'a.a. 2023/2024 al III anno del
Corso di Dottorato di ricerca Food, Health and Longevity, 37° Ciclo

consapevole del fatto che l'Università ha adottato un servizio *web-based* per rilevare il plagio,
attraverso un sistema software chiamato "Turnit.in",

consapevole delle sanzioni penali previste in caso di dichiarazioni non veritiere e di falsità negli atti
e della conseguente decadenza dei benefici di cui agli artt. 75 e 76 del D.P.R. 445/2000 e s.m.i.,

DICHIARA

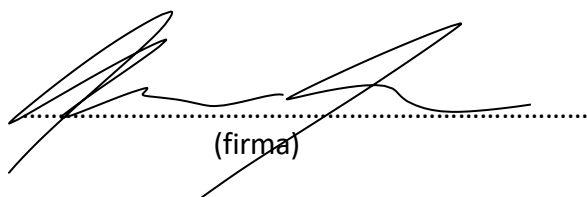
- di aver sottoposto la propria tesi di dottorato alla scansione di Turnit.in.

Ritiene che, dalla verifica, la Tesi è ragionevolmente risultata un documento originale, che cita
correttamente la letteratura.

È infine consapevole che la propria tesi di dottorato può essere verificata dal proprio Supervisore
e/o dal Coordinatore del Corso di dottorato per confermarne l'originalità.

Bruxelles, 03/02/2025

.....
(luogo e data)


.....
(firma)

To my parents, whose love and support
known no distance.

INDEX

Preface..... p.4

Section 1. Investigating the social determinants of healthy ageing: insights from the Novara Cohort Study (NCS) Pilot.....p.7

- Chapter 1: Introduction.....p.8

- Chapter 2: Rationale and design of the NCS.....p.15

- Chapter 3: Preliminary results from the NCS pilot.....p.23

- Chapter 4: Discussion.....p.30

Section 2. Exploring inequities in breast cancer screening access for Canadian-born Indigenous and immigrant populations in Ontario.....p.34

- Chapter 5: Introduction.....p.35

- Chapter 6: Materials and Methods.....p.46

- Chapter 7: Results.....p.51

- Chapter 8: Discussion.....p.66

Conclusion: The role of socioeconomic determinants in health outcomes.....p.73

Scientific articles

Acknowledgements

Bibliography

Preface

This thesis represents the results of my PhD studies, which have taken me across different continents, disciplines, and epidemiological and public health research areas. During these three years (2021-2024), I had the opportunity to explore the role of individual and global factors in shaping the aging process and influencing health outcomes. The experiences of these last three years have profoundly changed my perspectives on health inequalities, both personally and as a researcher.

I began my PhD at the University of Piemonte Orientale, where I had the opportunity to work on establishing an aging cohort study, The Novara Cohort Study (NCS), which aims to investigate the role of environmental, biological, genetic, and socioeconomic factors in the aging process. As I was working toward developing the pilot study, I became increasingly fascinated by how the complex interaction of these factors influenced the aging process and its trajectories.

To broaden my understanding of the influence of socioeconomic factors on health outcomes, I conducted part of my PhD as a Visiting Graduate Student at Western University, Canada. There, I started investigating the role of socioeconomic factors in colorectal cancer (CRC) screening uptake in the province of Ontario and the Territories. This research highlighted the role of socioeconomic factors on health disparities and how these are embedded within communities and societies. While investigating inequalities in access to CRC screening in the Territories, I learned about the social, economic, and political disadvantages faced by the Canadian Indigenous populations and realized that health inequalities are rooted in broader social contexts, historical legacies, and socioeconomic policies and not only the result of individual behaviours.

With this realization, while working on projects related to my PhD, I enrolled in a Master's in Global Health at Maastricht University, where I got the chance to explore how economic, environmental, and structural factors shape health outcomes on a global scale. This program has shown me that health is a complex system influenced by global forces such as economic policies, climate change, and social inequities. During this master's, I had the opportunity to return to Canada, this time to McMaster University, to investigate refugees' health and health economics policies. I also completed my research on socioeconomic inequalities and CRC screening uptake in Canada, which became my master's thesis.

Throughout these three years, I understood how health is not only an individual attribute but is strongly shaped by the economic, social, and environmental factors in which one lives. These factors must be considered while carrying out research and designing policies.

Thanks to these experiences, I obtained a traineeship as a Project Officer at the European Health and Digitalization Executive Agency (HaDEA), where I saw first-hand how health policies are implemented to benefit all State Members and citizens living in the EU.

As the result of my PhD studies, here I present two studies that examine how socioeconomic factors influence aging and health outcomes. The first section of the thesis presents the rationale, study procedures, and initial findings from the NCS pilot study, which assessed the feasibility and efficacy of the NCS protocol, including data collection in preparation for the broader implementation of the cohort study. In this study, we presented the socioeconomic, lifestyle characteristics, and health features of the pilot study participants and their representativeness of the general population. The second study investigates how health status, behaviours, and access to healthcare influence the uptake of breast cancer (BC) screening in Ontario, Canada. This study explores the disparities in BC screening

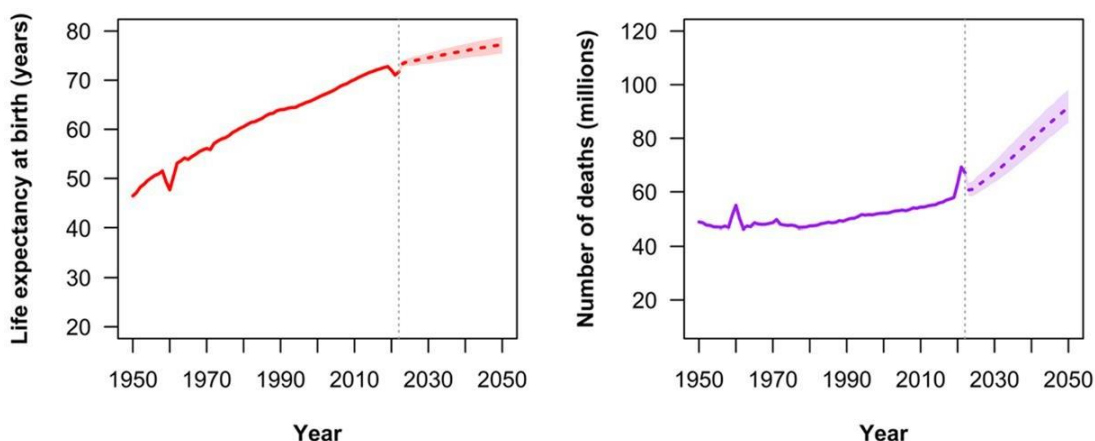
participation among Canadian-born, immigrant, and Indigenous populations, highlighting the role of socioeconomic and cultural factors in shaping access to preventive healthcare.

Section 1. Investigating the social determinants of healthy ageing: insights from the Novara Cohort Study (NCS) Pilot

1.1 Introduction

One of the main challenges that societies are facing in the 21st century is the rapid aging of the population, characterized by an increase in the number of years spent on disability (UN Population Division, 2020). Between 1990 and 2020, global life expectancy increased by more than 8 years, reaching 72.8 years in 2020. Projections indicate an increase in life expectancy, with a rise to approximately 77.2 years by 2050 (UN Population Division, 2022). Alongside, the population aged 60 years and older is projected to grow significantly, increasing from 1.1 billion in 2020 to 1.4 billion by 2030. The rapid growth and aging of the global population will be accompanied by an increase in the total number of deaths worldwide, rising from 67 million in 2022 to 92 million by 2050 (UN Population Division, 2022) (Figure 1).

Figure 1: Global life expectancy at birth (on the left) and number of deaths (on the right), estimates from 1950 to 2050. Adapted from UN Population Division, 2022

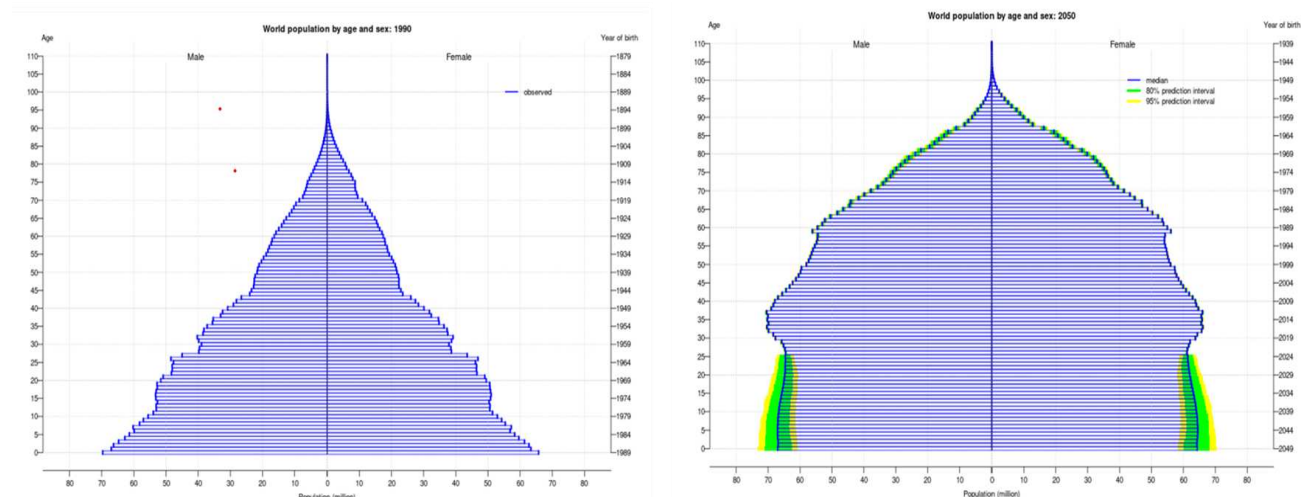


This societal aging is mainly driven by a reduction in fertility and longevity extension, reflecting the advances in public health, medical care, and economic development. These factors have led to a population distribution shift from younger to older (UN. Population Division, 2022). As highlighted by the United Nations (2020), the population over 60 is

increasing and is expected to rise dramatically worldwide in 2050 while the global fertility rate continues to decline (Figure 2).

Figure 2: Distribution of the world population by age and sex in 1990 (left panel) and in 2050 (right panel).

Adapted from the World Population Prospect, 2024, UN. Population Division

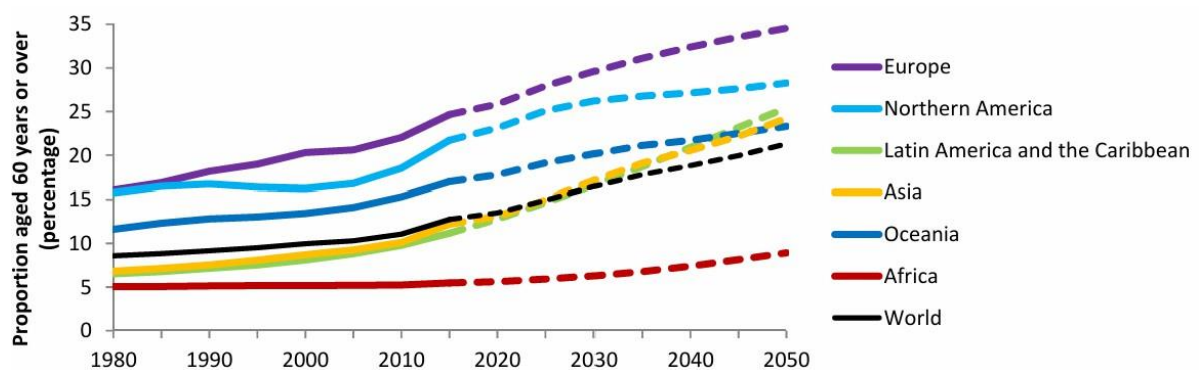


Global perspective

While aging is a global trend, its pace varies across regions. These variations are mainly due to demographic and epidemiological transition, fertility, mortality rates, and socioeconomic development. (UN. Population Division, 2022). In high-income countries (HIC), such as Europe, North America, and Australia, and a few Asian countries (e.g., Japan), populations are aging rapidly due to historically low fertility rates and high life expectancies (Chen et al., 2024). These areas have the highest proportion of older population, with 19% of the population aged 65 or older in 2022. In 2050, the population aged 65 or older is expected to reach 27%. Europe was the first region to undergo demographic transition, with today's European population being the most aged worldwide. Europe is projected to remain the most aged region in the upcoming decades, with 34% of the population projected to be 60 years or older in 2050, followed by North America (28%) (UN Population Division, 2017).

Low- and middle-income countries (LMICs) have younger populations but are undergoing demographic transition at a much faster pace compared to HICs. By 2050, two-thirds of the world's population aged 60 years and older will reside in LMICs, expected to outpace the East Asian nations at the population aging rate (Tan, 2022) (Figure 3).

Figure 3: Percentage of the population aged 60 or older, projected to 2050. Adapted from World Population Prospect: the 2017 Revision, UN. Population



Challenges and implications of an aging population

Although it is assumed that an increase in the health span accompanies an increase in longevity, this is often not the case. Evidence shows that the prevalence of severe and less severe disability among the older population is high and increasing worldwide. There is little to no evidence that older people today are in better health than the previous generation (WHO, 2015). This demographic shift from a predominantly younger to an older population presents significant challenges across multiple dimensions, including healthcare systems and economies.

A significant health concern linked to population aging is the increased incidence of non-communicable diseases (NCDs or chronic conditions) (Khan et al., 2024). NCDs “*tend to be of long duration and are the result of a combination of genetic, physiological, environmental, and behavioural factors*” (WHO, 2023). As individuals grow older, they are more susceptible to developing multiple NCDs. NCDs such as cardiovascular disease (CVD),

respiratory diseases, diabetes, obesity, cancer, and neurodegenerative disorders are the leading cause of death worldwide, accounting for 71% of all deaths (WHO, 2021). Among these, CVD remains the leading cause of death worldwide, followed by cancer, respiratory diseases, and diabetes. Approximately 80% of older adults are diagnosed with NCDs, such as obesity, diabetes, hypertension, or heart disease, and 50% have at least two diseases (WHO, 2021). These multimorbidities (defined as the coexistence of multiple two or more chronic diseases) significantly affect the conditions within the same individual (Cuschieri et al., 2024) and significantly strain the healthcare systems worldwide. For example, in the United States (US), NCDs contribute to over 75% of healthcare expenditures, accounting for over \$1.5 trillion in spending (Khan et al., 2024). In the EU, significant NCDs (CVD, cancer, diabetes, and chronic respiratory diseases) contribute 25% of healthcare expenditure and almost 2% of the GDP (Vandenberghe & Albrecht, 2019). This strain is particularly pronounced in low- and middle-income countries (LMICs), where healthcare resources and infrastructure are often insufficient to meet the growing demand caused by rapid demographic transitions (Tan, 2022).

Population aging also presents significant economic challenges, particularly regarding labour force participation and economic growth. The demographic transition to an older population is associated with a decline in labour force participation and savings rates (including pensions), raising concerns about future economic growth in the fastest-aging countries (OECD, 2023; Bloom et al., 2015). This decline is exacerbated by the increasing old-age dependency ratio, which financially burdens the working-age population to support retirees through social security systems (Hsu & Lo, 2019). This economic shift directly impacts healthcare systems by increasing the demand for medical services and long-term care while reducing the economic capacity to fund them, creating challenges for the sustainable maintenance of these fundamental systems.

Healthy aging and social determinants of health

While the aging population's challenges are considerable, they also emphasize the need to shift the focus from increasing life expectancy to improving the quality of life during aging. The WHO (2020) defines healthy aging as “*the process of developing and maintaining the functional ability that enables well-being in older age,*” which emphasizes the interaction between an individual’s intrinsic capacity and their environment. WHO, in its “UN Decade of Healthy Ageing: Plan of Action 2021-2030” (2020), highlights the possible implications of additional years of life. Older adults who experience these extra years in good health, remaining integral to societies and families, can strengthen societal cohesion and economic stability. Conversely, older adults who face poor health, social isolation, and/or dependency on care can contribute to a greater societal strain, including increased healthcare costs and reduced economic productivity.

It is important to highlight that healthy aging is influenced by factors that extend across the life course, including early life conditions, lifestyle choices, and access to healthcare and supportive environments (WHO, 2020). Aging and its trajectories are shaped not only by individual choices but are rooted in the broader context of social determinants of health (SDH) that accumulate over a lifetime, influencing health outcomes. SDH shapes the conditions in which individuals are born, grow, live, work, and age (WHO, 2019). Inequities experienced throughout life build cumulatively, leading to advantages or disadvantages that manifest more acutely in later years (WHO, 2015). These factors operate within an interconnected framework, where social determinants interact to create pathways that either enhance or hinder healthy aging.

Socioeconomic status (SES) plays an important role in determining access to resources such as safe housing, quality healthcare, and other resources. Individuals with

lower SES (income, education, social class) often face disadvantages, which can lead to poorer health outcomes later in life (Glymour et al., 2014). Studies indicate that individuals with lower SES have, on average, poorer health compared with those with higher SES (Darin-Mattsson et al., 2017). However, the influence of SDH extends beyond socioeconomic factors, including systemic and structural inequalities that are present in various aspects of life. For instance, disparities in access to transportation or safe public spaces can limit opportunities for older adults to remain active and engaged (MacLeod et al., 2022).

The multifaceted nature of SDH highlights the importance of using an integrated approach to promoting healthy aging. Rather than addressing individual determinants, interventions must focus on addressing systemic inequities and support holistic well-being. Policies aimed at improving housing, transportation, education, and social support not only enhance health outcomes but also foster equity, strengthening the social fabric. By reducing barriers to access and nurturing environments that promote equity, societies can mitigate the long-term effects of adverse social determinants and support healthy aging initiatives.

Investigating Ageing and the Novara Cohort Study

The complex interplay between SDH and aging trajectories highlights the need for comprehensive, population-based studies to better understand these dynamics. While it is well established that SDH, such as socioeconomic status, education, and access to healthcare, shape health outcomes, their cumulative effects on aging remain less understood. These social factors, alongside regional, cultural, and environmental influences, interact with biological determinants such as genetic predispositions and molecular aging processes, creating complex pathways that affect individual aging trajectories. Epidemiological studies are essential to investigate these interactions and address factors influencing healthy and unhealthy aging.

Several epidemiological cohorts have been established in the last decades to examine the burden of NCDs and the population's aging. Some cohorts have focused on investigating specific types of disease, some on particular age ranges, and others have limited data due to their large size (questionnaire-based cohort studies). The Novara Cohort Study (NCS), a population-based study with a longitudinal design, uses a multidisciplinary approach to studying aging, considering all the factors possibly involved in the aging process. For its interdisciplinary approach, the NCS was established in collaboration with the UPO-Biobank, infrastructure of the University of Piemonte Orientale created to collect and store biological samples and associated data, the “Maggiore della Carità” Hospital in Novara and the Territorial Health Care Service in Novara (ASL13 Novara). These collaborations guarantee continuous interaction with the local population, raising awareness of a more conscious approach to health. The NCS was designed to gather a wide range of data, including physical function, well-being, mental health, quality of life, medical history, and biological samples. These data provide essential information on the population's health status and are critical for the stakeholders and policymakers in designing interventions for an aging population.

Here, the rationale, methodology, and preliminary findings of the Novara Cohort Study (NCS) pilot, completed in January 2024, are presented. The pilot assessed the feasibility of the study's methodology and examined the baseline characteristics and representativeness of the enrolled population.

1.2 Rationale and design of the Novara Cohort Study and its pilot study

Study objectives

The Novara Cohort Study was designed with the aim to:

1. Identify determinants of healthy and unhealthy aging and describe the health status of the population living in Novara province
2. Collaborate with policymakers and stakeholders to design interventions to improve the quality of life and health in aging
3. Involve the general population in scientific research, raising awareness towards improving quality of life in aging and prevention of chronic disease
4. Proof of concept for identifying molecular markers of accelerated aging

The NCS pilot study was specifically designed to assess the feasibility and practicality of the study's methodology, including participant recruitment, data collection, and protocol adherence while gathering preliminary data to refine the full-scale study design.

Study design and participants

The NCS is a longitudinal population study aiming to include a minimum of 10,000 participants aged 18 or older, representing Novara Province residents. Situated in northwest Italy within the industrialized region known as the 'Pianura Padana,' Novara Province spans 88 municipalities and is positioned between two of Italy's largest and most polluted metropolitan urban areas of the country, Milan and Turin. As of January 2023, the Novara Province had 362,502 residents, with a demographic breakdown of 48.8% male and 51.2% female, an average age of 46.9 years, and an aging index of 201. Approximately 25% of the population is over 65 years old, including 8% who are over 80 years old.

Recruitment process

In the study's first phase, the Novara province population over 35 was invited to participate through informative and mass media campaigns. To reach the entire population, NCS investigators have presented the NCS and its objectives to the general population, GPs, other healthcare professionals, and municipality councils. Different public meetings were held where the study was presented to the population. These meetings were a crucial opportunity to involve the public in scientific research discussions. In the study's second phase, a letter of invitation or a phone call will be sent to random people in the eligible population identified through the Territorial Health Care Service in Novara (ASL13 Novara) lists. This will ensure that the population study is representative of the target population, allowing for statistical inference.

Participants were stratified into two age categories: 35-64 and over 65. Based on the participant's age, active follow-ups will be carried out every 5 years for participants under 65 and every 3 years for participants over 65. During active follow-up, participants will repeat questionnaires completed during the baseline assessment. Passive follow-ups will be based on the linkage between administrative data and health and death registries upon consent from the participant. This follow-up strategy will allow NCS to collect participant data to assess their health status.

Pilot study enrolment and procedure

The pilot study was carried out between November 2022 and January 2024 and included a total of 123 participants. These underwent a comprehensive assessment, which included collecting biological samples, questionnaires, medical examinations, and functional and cognitive tests, with the entire process taking up to four hours. The assessment team consisted of a medical doctor, a nurse practitioner, and three researchers of the NCS staff. The testing center was in Novara, within the University of Piemonte Orientale (UPO)

research biobank (UPO Biobank) rooms. The UPO Biobank outpatient clinic and laboratories ensured adherence to ethical and quality standards for all protocol procedures and the collection of high-quality biological samples and data.

Questionnaire data collection

All participants, after the informed consent procedure, complete a self-administered questionnaire reporting:

- 1) Lifestyle, physical activity, and sleep: information about smoking habits, exposure to tobacco smoking, use of electronic cigarettes, and alcohol consumption habits were collected in the lifestyle questionnaire along with information about social network usage and cultural activities (“How many times in a year do you go to the movies/theatre or art exhibitions?”). Information about physical activity was collected using the International Physical Activity Questionnaire (IPAQ) (Hagströmer et al., 2006). The questionnaire focused on the physical activity carried out during working hours and outside the working place (physical activity was ranked as light, moderate and intense). Information about sleeping habits was collected using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) and the Epworth Sleepiness Scale (ESS) (Johns, 1991). This questionnaire collects information about snoring frequencies, nocturnal apnoea, daytime sleepiness, frequency of unrested sleep, and the presence of OSAS.
- 2) Diet: General information about diet was collected with a modified version of the EPIC questionnaire (Riboli et al., 2002). The questionnaire focused on the frequency and quantity of meat (red and white), vegetables/fruits, and carbohydrates consumed monthly. It also included photos of each food category that estimated the quantity of food eaten in every portion.

- 3) Quality of life: A novel questionnaire was developed to assess quality of life. It collected information about life satisfaction, physical and psychological health, social behaviour, and sexual satisfaction.
- 4) Mental health: current level of depression, anxiety, and attachment style was measured using the Beck Depression Inventory-II (BDI) (Beck et al., 1996), Beck Anxiety Inventory (BAI) (Beck et al., 1988) respectively,) and Attachment Style Questionnaire (Feeney & Ryan, 1994).)

Furthermore, participants were also interviewed by a healthcare professional involved in the NCS pilot study regarding:

- 5) Demographic data: information about age, sex, place of birth, ethnicity, education level, marital status, occupational history (and occupational exposure), and family history were collected.
- 6) Medical history: information on the current use of prescription or non-prescription medications and supplements was collected along with medical history, including the age of diagnosis of different chronic diseases (i.e., cardiovascular, endocrine, mental health, neurological disorder, cancer diagnosis, autoimmune diseases, diabetes, hepatic cirrhosis, chronic kidney diseases);
- 7) Family medical history: Participants were asked for information on the age of each participant's biological parents and siblings and the cause of death (if deceased). A family history of common medical conditions (chronic diseases) was also collected.
- 8) Cognitive functions: Each participant's cognitive function was evaluated through the Montreal Cognitive Assessment (MOCA), a scoring system with high sensitivity and specificity in detecting early mild cognitive impairment (Nasreddine et al., 2005). A healthcare provider administered the test.

A healthcare professional involved in the study assessed the participant's frailty status using the Clinical Frailty Scale (CFS) (Rockwood, 2005).

Functional status

The functional status of NCS participants was assessed using:

- 1) Handgrip test: used to assess physical fitness and frailty in the elderly.
- 2) Timed up-and-go test: This test estimates the risk of falls, especially in older adults and community-dwelling people. Physicians often use it as a screening tool (Nightingale et al., 2018).
- 3) The 6-minute walking test estimates cardiorespiratory fitness and is strictly related to cardiovascular risk and all-cause mortality (Kammin, 2022).
- 4) Short Physical Performance Battery (SPPB): This scale explores the reduction of physical performance in older persons, particularly muscle strength of lower extremities and balance (Western & Malkowski, 2022).

These measures were used to assess the general physical fitness of the participants in the study.

Anthropometric data

During the physical assessment, two healthcare professionals (a medical doctor and a nurse) collected information about height, weight, body mass index (BMI), waist circumference, waist-to-hip ratio (WHR), blood pressure, heart and respiratory rate, and blood oxygen saturation.

Biological sample collection and biomarkers

Blood, saliva, and urine samples were collected, processed, and stored in UPO Biobank. Participants autonomously collected their saliva and urine samples while a trained

nurse collected the blood samples following standardized procedures. Blood collection occurred after the participants had fasted overnight. Upon completion of the biospecimen collection, breakfast was offered to all participants. Approximately 50 ml of blood was drawn from each participant, using a variety of tubes to accommodate different analyses and storage requirements (Table 1): i) Ethylenediaminetetraacetic acid (EDTA) tubes, used for fresh hematological analyses, as well as for biobanking dedicated to nucleic acid extractions, peripheral blood mononuclear cells (PBMC) cryopreservation, and plasma proteomic and metabolomic analyses; ii) Lithium Heparin (LH) tubes, for plasma dedicated to biochemical analyses and biobanking; iii) Na-Citrate-containing tubes, for coagulation tests; iv) Gel serum separator tubes, for easy separation of serum from the clot, dedicated to serological and immunological analyses. A portion of the fresh blood samples was immediately subjected to a series of hematological and biochemical assessments. The panel includes biomarkers associated with electrolyte balance, inflammation, cardiovascular disease risk, and indicators of renal damage, bone marrow, thyroid, and liver function (Table 1). Participants received the results of these tests and guidance on how to discuss them with their general practitioner.

Table 1. Biospecimens: collection tubes, volumes, blood analyses on fresh samples, and biobanking

Collection tubes	Volume (ml)	Material	Blood biomarkers on fresh samples	Biobanking		
				N° of aliquots	Aliquots volume (µl)	Storage temp. (°C)
Serum gel (BD Vacutainer)	15	Serum	CYSC, protein electrophoresis, TP	4	500	-180
K2-EDTA (BD Vacutainer)	16	EDTA plasma	WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PDW, MPV, P-LCR, PLT, NEU, LYMPHO, MONO, EOS, BASO, EB, IG, HbA1c	6	500	-180
			EDTA buffy coat	1	300	-80
			Whole blood in RNA later	2	1600	-20
			PBMC	2	500 (2.0 mln)	-180
Sodium-Citrate (BD Vacutainer)	3	Citrate plasma	FBG, DD	4	500	-180
		Citrate buffy coat		2	300	-80
Gel Lithium-Heparin (BD Vacutainer)	13.5	Heparin plasma	ALT, AST, BIL, BUN, Ca, TC, CR, FER, FA, ALP, P, GGT, HDL, IL-6, LDL, CRP, K, Na, FT4, TRF, TG, TSH, UA, B12	6	500	-180
Vacutest (Securlab)	80-100	Urine		10	5000; 500	-80
Salivette (Sarstedt)	0.5	Saliva				-80

ALT, Alanine Aminotransferase; *ALP*, Alkaline Phosphatase; *AST*, Aspartate Aminotransferase; *BASO*, Basophils; *BIL*, Bilirubin; *BUN*, Blood Urea Nitrogen; *CA*, Calcium; *CR*, Creatinine; *CRP*, C-Reactive Protein; *CYSC*, Cystatin C; *DD*, D-dimer; *EB*, Erythroblasts; *EOS*, Eosinophils; *FA*, Folic Acid; *FBG*, Fibrinogen; *FER*, Ferritin; *FT4*, Free-thyroxine (FT4); *GGT*, Gamma-Glutamyl transferase; *HbA1c*, Glycated Hemoglobin; *HDL*, High-Density Lipoprotein; *HCT*, Hematocrit; *HGB*, Hemoglobin; *IG*, Immature Granulocytes; *IL-6*, Interleukin-6; *K*, Potassium; *LDL*, Low-Density Lipoprotein; *LYMPHO*, Lymphocytes; *MCV*, Mean Corpuscular Volume; *MCH*, Mean Corpuscular Hemoglobin; *MCHC*, Mean Corpuscular Hemoglobin Concentration; *MONO*, Monocytes; *MPV*, Mean Platelet Volume; *Na*, Sodium; *NEU*, Neutrophils; *P*, Phosphorus; *PDW*, Platelet Distribution Width; *PLT*, Platelets; *P-LCR*, Platelet Larger Cell Ratio; *RBC*, Red Blood Cells; *RDW-CV*, Red Cell Distribution Width-Coefficient of Variation; *RDW-SD*, Red Cell Distribution Width-Standard Deviation; *TSH*, Thyroid Stimulating Hormone; *TP*, Total Proteins; *TRF*, Transferrin; *UA*, Uric Acid; *WBC*, White Blood Cells.

Data collection and analysis

The study utilized REDCap version 14.0.33 for secure pseudonymized data storage. Descriptive analyses presented categorical variables as counts and percentages, while continuous variables were reported using mean and standard deviations (SD) for normally

distributed variables or median and interquartile ranges (IQR) for skewed variables. Pearson's chi-square test was used to verify associations between categorical variables. NCS pilot demographic and lifestyle characteristics were compared to those obtained from the PASSI (Progressi delle Aziende Sanitarie per la Salute in Italia) surveillance system (ISS, 2014). PASSI employs a random sampling technique for collecting information about lifestyle and risk factors for non-communicable diseases (NCDs) among the general adult population. For this study, data from residents in the Local Health Authority (LHA) of Novara between 60-69 years old were retrieved. Statistical analyses were performed using STATA version 17. All tests were two-tailed, with a type I error set to 0.05.

Ethical considerations and privacy

The local Ethical Committee approved the study protocol (Comitato Etico Interaziendale AOU Maggiore della Carità di Novara, Protocol Number CE137/2022). Participants were required to provide informed consent, ensuring their voluntary participation and understanding of the study's purpose and procedures. All data collection and management procedures adhere to the guidelines outlined in the EU General Data Protection Regulation (GDPR) 2016/679.

1.3 Preliminary results from the NCS pilot

Demographic and lifestyle characteristics of NCS pilot study participants

This section provides an overview of the results of the NCS pilot study. This pilot aimed to evaluate the feasibility of recruitment methods, data collection processes, and participant engagement while also gathering preliminary data on the cohort's demographic and health characteristics. An overview of the NCS pilot participants' demographic characteristics is provided in Table 2. The NCS pilot study included 123 participants, 68 (55.3%) women and 55 (44.7%) men; 45.5% were under 65, while 54.5% were 65 or older. Most participants had an upper secondary school diploma, with 45.4% of men and 55.9% of women receiving this qualification. Most participants were married (men 80%, women 58.8%), living with someone (men 85.5%, women 79.4%), and retired (men 67.3%, women 66.2%).

Lifestyle characteristics and risk factors are presented in Table 3. Most participants identified themselves as either non-smokers or former smokers (76.4% of men and 95.6% of women). Almost all identified as omnivorous (92.7% of men and 89.7% of women). Adherence to a vegetarian diet was reported by 5.9% of women. Regarding alcohol consumption, the majority of NCS pilot participants (70.9% of men and 82.3% of women) reported consuming less than one alcoholic unit daily. Data on physical activity using the IPAQ questionnaire indicated that most of the NCS pilot population was at least moderately active (71.0% of men and 64.7% of women). The Pittsburgh Sleep Quality Index (PSQI) revealed that most NCS pilot participants experienced poor sleep quality (56.4% of men and 60.3% of women).

Table 2. NCS pilot participant demographic characteristics

	NCS participants		
	Men n=55	Women n=68	Total n=123
Age (years)-mean (SD)	65.5 (10.0)	64.7 (8.1)	65.1 (9.0)
Age ≤65			
Mean (SD)	56.5 (7.2)	58.8 (6.1)	57.9 (6.6)
Median (min-max)	57 (44-65)	61.5 (36-65)	61 (36-65)
Age >65			
Mean (SD)	72.0 (5.7)	71.4 (4.0)	71.7 (4.9)
Median (min-max)	71 (66-93)	71 (66-80)	71 (66-93)
	n (%)	n (%)	n (%)
Education			
Primary or lower middle school	9 (16.4)	9 (13.2)	18 (14.7)
High school	25 (45.4)	38 (55.9)	63 (51.2)
University degree	21 (38.2)	21 (30.9)	42 (34.1)
Marital status			
Single, divorced or widower	10 (18.2)	27 (39.7)	37 (30.1)
Married	44 (80.0)	40 (58.8)	84 (68.3)
Missing	1 (1.8)	1 (1.5)	2 (1.6)
Cohabitation status			
Living alone	8 (14.5)	14 (20.6)	22 (17.9)
Cohabiting	47 (85.5)	54 (79.4)	101 (82.1)
Employment status			
Employed	18 (32.7)	15 (22.0)	33 (26.8)
Unemployed	0 (0.0)	8 (11.8)	8 (6.5)
Retired	37 (67.3)	45 (66.2)	82 (66.7)

n, number; *SD*, standard deviation.

Table 3. NCS pilot participant lifestyle and risk factors

NCS participants			
	Men n=55	Women n=68	Total n=123
	n (%)	n (%)	n (%)
Smoking habits			
Never smoker	21 (38.2)	34 (50.0)	55 (44.7)
Past smoker	21 (38.2)	31 (45.6)	52 (42.3)
Current smoker	9 (16.3)	2 (2.9)	11 (8.9)
Missing	4 (7.3)	1 (1.5)	5 (4.1)
Alcohol consumption			
Never to seldom	11 (20.0)	23 (33.8)	34 (27.6)
<1 alcohol unit/day	28 (50.9)	33 (48.5)	61 (49.6)
≥1 alcohol unit/day	9 (16.4)	7 (10.3)	16 (13.0)
Missing	7 (12.7)	5 (7.4)	12 (9.8)
Physical activity			
Low	11 (20.0)	19 (27.9)	30 (24.4)
Moderate	23 (41.9)	27 (39.7)	50 (40.7)
High	16 (29.1)	17 (25.0)	33 (26.8)
Missing	5 (9.1)	5 (7.4)	10 (8.1)
Participation in screening programs			
No	25 (45.5)	2 (2.9)	27 (21.9)
Yes	30 (54.5)	66 (97.1)	96 (78.1)
BMI			
< 25	28 (50.9)	33 (48.6)	61 (49.6)
25-29.99	20 (36.4)	24 (35.3)	44 (35.8)
≥ 30	7 (12.7)	10 (14.6)	17 (13.8)
Missing	-	1 (1.5)	1 (0.8)
Diet			
Omnivorous diet	51 (92.7)	61 (89.7)	111 (90.2)
Vegetarian diet	0	4 (5.9)	4 (3.3)
Other	1 (1.8)	1 (1.5)	2 (1.6)
Missing	3 (5.5)	2 (2.9)	5 (4.1)
Sleep Quality			
Poor	31 (56.4)	41 (60.3)	72 (58.6)
Good	18 (32.7)	24 (35.3)	42 (34.1)
Missing	6 (10.9)	3 (4.4)	9 (7.3)

n, number; *BMI*, Body Mass Index

Health status of the NCS pilot study participants

Common cardiovascular disease (CVD) risk factors were evaluated (Table 3 and Table 4). Almost one in two participants was overweight ($BMI \geq 25$), in particular 49.1% of men and 49.9% of women. The average systolic blood pressure (BP) recorded during the visits was 122.9 (13.6) mmHg for men and 123.8 (15.8) mmHg for women, both slightly above the recommended level (<120 mmHg). In contrast, diastolic BP remained within the normal range (<80 mmHg), averaging 76.4 mmHg for men and 77.3 mmHg for women.

Table 4. Blood pressure, self-reported NCD, OTC, and prescription medications

	NCS participants		
	Men n=55	Women n=68	Total n=123
Blood pressure - mean (SD)			
Systolic BP (mmHg)	122.9 (13.6)	123.8 (15.8)	123.3 (14.8)
Diastolic BP (mmHg)	76.4 (9.0)	77.3 (10.5)	76.9 (9.8)
Self-reported diagnosis of NCD (excluding hypertension and hypercholesterolemia)			
	n (%)	n (%)	n (%)
0	13 (23.6)	20 (29.4)	33 (26.8)
1	22 (40.0)	19 (27.9)	41 (33.3)
2-3	12 (21.8)	18 (26.5)	30 (24.4)
≥ 4	8 (14.6)	11 (16.2)	19 (15.5)
Self-reported number of OTC and prescription medications			
	n (%)	n (%)	n (%)
None	16 (29.1)	11 (16.2)	27 (22.0)
1-4	32 (58.2)	49 (72.1)	81 (65.9)
≥ 5	7 (12.7)	8 (11.8)	15 (12.2)

NCD, non-communicable diseases; *OTC*, over the counter; *n*, number; *SD*, standard deviation; *BP*, blood pressure.

Among the participants, 29.1% of men and 42.6% of women reported a diagnosis of hypertension. Despite all of them claiming full adherence to antihypertensive therapy, elevated blood pressure readings were found in 47.0% of men and 55.2% of women during

the visit. On the other hand, of those who did not report having hypertension, 42.2% of men and 41.0% of women still exhibited blood pressure readings above the normal range. Forty percent of men and 27.9% of women reported having one chronic disease, while 36.4% of men and 42.7% of women declared having two or more conditions. The most frequent conditions were endocrine and metabolic diseases (34.5% of men and 57.4% of women), followed by cardiovascular disease (30.9% of men and 25% of women) and neoplasia (16.4% of men and 22.1% of women). Osteoporosis was reported by 1.8% of men and 29.4% of women.

Women reported a higher (83.9%), albeit not statistically significant, use of over-the-counter (OTC) and prescription medications compared to men (70.9%). The most used prescribed drug classes were antihypertensives (27.3% of men and 35.3% of women), endocrine therapy (14.5% of men and 22.1% of women), lipid-lowering drugs (10.9% of men and 11.8% of women), antithrombotic drugs (16.4% of men and 10.3% of women), and beta-blockers (10.9% of men and 10.3% of women).

Representativeness of the NCS pilot population compared to the general population

To evaluate the representativeness of the NCS pilot sample, we compared the demographic and lifestyle characteristics of the NCS pilot study participants with those of residents aged 60-69 in the ASL Novara, as derived from the PASSI surveillance system. Compared with data from PASSI, the NCS pilot participants had a significantly higher level of education ($p < 0.001$). Specifically, only 14.7% of NCS participants had primary or lower middle school education compared to 55.5% in PASSI; 51.2% had high school education compared to 34.9% in PASSI; and 34.1% had a university degree compared to 9.6% in PASSI. In terms of smoking habits, the NCS pilot participants exhibited a significantly lower prevalence of current smokers (8.9% vs. 17.0%, $p = 0.04$). Alcohol consumption data showed

that NCS pilot participants drank more alcohol, with 13.0% consuming ≥ 1 alcohol unit/day compared to 5.2% in PASSI ($p=0.009$). Conversely, those consuming <1 alcohol unit/day were more prevalent in PASSI than in the NCS pilot (77.2% vs 94.8%). Physical activity levels revealed a significantly higher proportion of individuals with low physical activity in the PASSI group (45.1%, $p<0.001$) compared to the NCS pilot group (24.4%). Moderate activity levels showed no significant difference between the groups (40.7% in NCS vs. 32.4% in PASSI), nor did high activity levels (26.8% in NCS vs. 22.5% in PASSI). There were no significant differences between the NCS pilot and PASSI data regarding marital status, cohabitation, employment status, participation in cancer screening programs, or BMI category distribution. However, some variations were observed, which may reflect differences in sample composition or the influence of sample size on statistical power.

Table 5. Representativeness of the NCS Pilot cohort compared to the general population

	NCS pilot	PASSI	<i>p-value</i>
	n=123	n=218	
Age (years) - mean (SD)	65.1 (9.0)	64.0 (2.92)	0.190
	n (%)	n (%)	
Education			
Primary or lower middle school	18 (14.7)	121 (55.5)	<0.001
High school	63 (51.2)	76 (34.9)	<0.001
University degree	42 (34.1)	21 (9.6)	<0.001
Marital status			
Single, divorced or widower	37 (30.1)	53 (24.3)	0.250
Married	84 (68.3)	165 (75.7)	0.250
Missing	2 (1.6)		
Cohabitation status			
Living alone	22 (17.9)	27 (12.4)	0.160
Cohabiting	101 (82.1)	191 (87.6)	0.160
Employment status			
Employed	33 (26.8)	57 (26.1)	0.890
Unemployed	8 (6.5)	7 (3.2)	0.150
Retired	82 (66.7)	152 (69.7)	0.550
Smoking habits			
Never smoker	55 (44.7)	106 (48.6)	0.487
Past smoker	52 (42.3)	75 (34.4)	0.132
Current smoker	11 (8.9)	37 (17.0)	0.040
Missing	5 (4.1)	-	-
Alcohol consumption			
Never to seldom	34 (27.6)	82 (38.1)	0.060
<1 alcohol unit/day	61 (49.6)	125 (56.7)	0.168
≥1 alcohol unit/day	16 (13.0)	11 (5.2)	0.009
Missing	12 (9.8)	-	-
Physical activity			
Low	30 (24.4)	96 (45.1)	<0.001
Moderate	50 (40.7)	69 (32.4)	0.090
High	33 (26.8)	48 (22.5)	0.316
Missing	10 (8.1)	-	-
Participation in cancer screening programs			
No	27 (21.9)	37 (17.0)	0.258
Yes	96 (78.1)	181 (83.0)	0.258
BMI			
< 25	61 (49.6)	105 (48.4)	0.800
25-29.9	44 (35.8)	84 (38.7)	0.613
≥ 30	17 (13.8)	28 (12.9)	0.798
Missing	1 (0.8)	-	-

n, number; *SD*, standard deviation; *BMI*, body mass index

1.4 Discussion

The NCS represents an important endeavour in the field of aging research, as it aims to comprehensively investigate both cross-sectional and longitudinal aspects of aging within the Novara population. By examining multiple dimensions of aging, including socioeconomic, environmental, physical, cognitive, biological, and psychological aspects, the NCS aims to help elucidate the complex interplay of factors influencing aging trajectories and outcomes and inform evidence-based interventions and strategies to promote well-being and longevity. The pilot study has demonstrated the feasibility of scaling the NCS to a larger cohort. It tested recruitment strategies, data collection methods, and participant engagement processes, providing insights into logistical and methodological challenges. These results confirm the ability to implement a larger longitudinal investigation while identifying possible areas for refinement, such as questionnaires, improving public engagement strategies, and addressing cohort representativeness. The longitudinal follow-ups will allow researchers to evaluate participants' changes in health outcomes, functional abilities, lifestyle factors, and environmental exposures as they age. In particular, the NCS pilot has been instrumental in identifying critical areas for improvement across all phases of the NCS, including public engagement, participant procedures, and the types of questionnaires and functional tests administered. This study also aimed to describe the baseline characteristics of the population enrolled in the pilot study and evaluate its representativeness. This was assessed by comparing the characteristics of the NCS Pilot population with a random sample of the Province of Novara, the study's area. The sample is represented by citizens aged 60-69 years contacted by the PASSI Survey (ISS, 2014), a national study to assess the health status of the Italian population. Compared with the data from PASSI, the NCS pilot participants were more likely to have a higher level of education ($p < 0.001$). Additionally, NCS pilot participants demonstrated a lower prevalence of low physical activity levels ($p < 0.001$),

indicating a more active lifestyle overall. Notably, no significant differences were found in moderate ($p=0.09$) and high ($p=0.316$) physical activity levels. Moreover, there was a statistically significant higher prevalence of consumption of one or more alcohol units per day in the NCS pilot participant when compared with participants from PASSI ($p=0.01$).

While the NCS pilot's successful recruitment was mostly voluntary, it is fundamental to acknowledge the limitations and biases that this recruitment method can lead to. Compared with the general population, the NCS pilot participants were found to have a significantly higher level of education and a more active lifestyle. This outcome is common in research studies, particularly those involving voluntary participation, where a higher proportion of participants with higher education levels is often observed. This phenomenon, known as self-selection bias or volunteer bias, occurs when individuals with certain characteristics, such as higher education levels, are more likely to volunteer for research studies (Popovic & Huecker, 2023). Volunteers may exhibit exposure or outcomes that differ from those of the general population, possibly causing the association of interest to differ from the true association present in the general population, threatening the study's internal validity (Van Alten et al., 2024). Bias in an association can occur when characteristics influencing participation also affect the outcome of interest. For example, factors contributing to this trend, such as socioeconomic status, health literacy, access to information, healthcare, or motivation to participate in health-related research, can lead to the overrepresentation of certain groups, leading to false positive associations and/or associations of the incorrect sign (Van Alten et al., 2024). Additionally, structural factors like geographic access to study sites, cultural attitudes toward research, and the availability of resources contribute to discrepancies between the study population and the general population. Volunteers might have a different perception of the study's relevance or saliency (e.g., time or financial stability), further contributing to biased associations (Jordan et al., 2013).

Participants in the NCS pilot were volunteers, showing interest in contributing to research and scientific progress. Different studies show that higher education levels often correlate with increased health consciousness and proactive health management (Raghupathi & Raghupathi, 2020). Individuals with such backgrounds are more likely to engage in health-related research, seeking to understand their health, advance scientific knowledge better, and potentially benefit from health interventions. Moreover, those with higher education typically exhibit a strong interest in learning and contributing to academic or scientific pursuits, further motivating their involvement in research aligned with their expertise or interests (Jordan et al., 2013). Many studies highlight similar findings: compared to the general population, the population included in studies through voluntary participation is significantly different. Studies found that these participants are older, female, less urban, better educated, white, have higher income, live in well-off areas, are up-to-date with screening and doctor's visits, and require fewer medications (Brayne & Moffitt, 2022). A study by Van Alten et al. (2024) demonstrated that while volunteer bias was of little importance when assessing risk factors for mortality, it matters more substantially when investigating socioeconomic and health-related variables. These studies demonstrate that striving for a representative sample is crucial. Using strategies to alleviate volunteer bias will allow us to identify individuals with different educational levels and socio-demographic backgrounds. This approach will increase the reliability of the NCS findings. Such awareness urges us to devise strategies such as randomized calls or invitations to promote inclusivity and accessibility in research participation, ensuring that study outcomes are relevant and beneficial to the general population.

Volunteers allowed us to achieve good engagement with the Novara community in the research process. This was achieved through a series of public engagement events, including open days and public meetings, which allowed citizens to interact directly with the research

team involved in the NCS. Such events were fundamental to fostering a sense of trust between the Novara community and the researchers. Trust, as highlighted by Wilkins (2018), is crucial in cohort studies as it encourages engagement and active participation by the citizens. Trust in researchers and the belief in the research contribute significantly to the population's willingness to participate in cohort studies (Wilkins, 2018). Moreover, engaging with participants in longitudinal studies has been shown to promote their recruitment and retention throughout the study (Ochieng et al., 2021). Such events were crucial not only for the success of the NCS pilot study but also for establishing long-lasting relationships between the participants and the NCS research team. These relationships are fundamental to the success of any cohort study, particularly to maintaining retention and sustainability of the study (Teague et al., 2018).

In conclusion, the Novara Cohort Study (NCS) pilot has been instrumental in evaluating the feasibility of conducting a larger-scale study on aging. The pilot successfully engaged the local population and collected comprehensive data; challenges like selection bias highlight the need for more inclusive recruitment strategies in future waves. These findings lay the groundwork for longitudinal research that can inform public health policies and interventions promoting healthy aging. By addressing identified limitations, the NCS has the potential to significantly contribute to understanding complex factors influencing health and improving health aging trajectories in aging populations.

Section 2. Exploring inequities in breast cancer screening access for Canadian-born Indigenous and immigrant populations in Ontario.

2.1 Introduction

Breast cancer (BC) is a leading cause of morbidity and mortality, contributing significantly to the global burden of disease (Trapani et al., 2023). It is the second most commonly diagnosed cancer worldwide, with approximately 2.3 million new cases and over 600,000 deaths reported in 2020, accounting for nearly 12% of all cancers diagnosed and 16% of cancer-related deaths in women (Bray et al., 2024). In 2019, the disease burden of BC has been quantified in a loss of 20,625,313 disability-adjusted life-years (DALYs), reflecting its significant impacts on societies and health systems (Vos et al., 2020).

Multiple lifestyle and non-modifiable risk factors are linked to a higher risk of BC, including increased older age, at least one first or second-degree relative diagnosed with BC, and heterogeneous or very dense breast tissue. Moreover, the risk for primary BC is higher in women with previous high-risk BC lesions, family history of breast and ovarian cancer, positivity for genetic markers associated with a high-risk BC, previous large doses of chest radiation before the age of 30, and type II diabetes. Modifiable risk factors include use of hormone replacement therapy, diet content (high fat and low fibres), smoking, low physical activity, increased BMI, alcohol use, nulliparity, or advanced age at first full-term delivery (Henderson et al., 2024)

Global trends and disparities

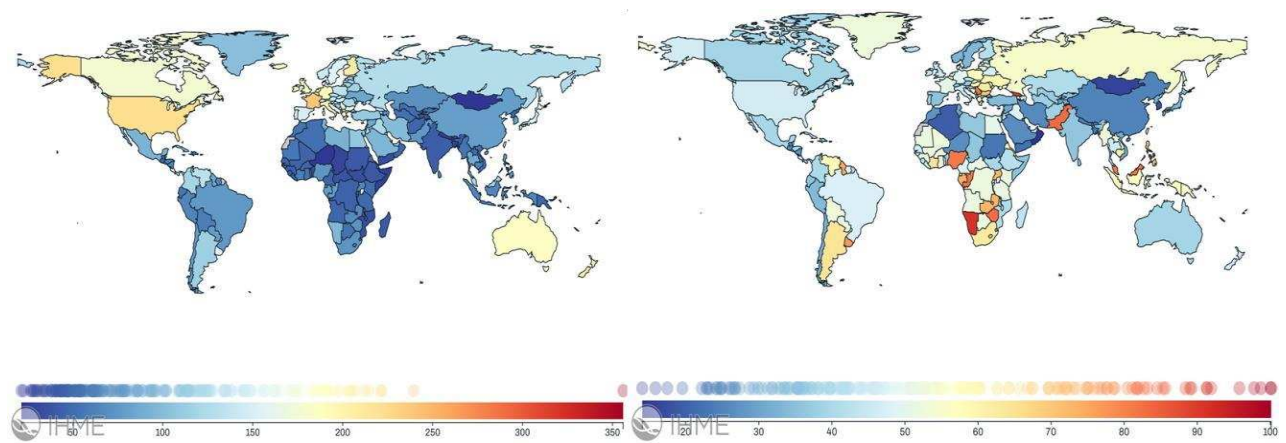
The incidence, prevalence and mortality for BC are not evenly distributed worldwide. BC incidence and mortality rates continue to rise globally, with notable differences observed across regions and age groups (Heer et al., 2020). In North America and Europe, the incidence of BC is at 97.0 and 83.3 per 100,000 individuals, respectively, whereas incidence rates are significantly lower in Africa and East Asia, at 27.0 per 100,000 (Loibl et al., 2021). HICs, mainly in Europe, have achieved an average of 40% reduction in BC mortality since

1990, primarily due to organized screening programs, early-stage diagnosis and timely treatment (Zielonke et al., 2020). However, in low-income (LICs) and lower-middle-income countries (LMICs), mortality rates are increasing, with estimates ranging from 17.4 to 20.1 per 100,000 (Lima et al., 2021). The 5-year survival rates in HICs exceed 90%, compared with 66% in India and 40% in South Africa (WHO, 2023). This disparity highlights the inequities in healthcare infrastructure in LICs and LMICs, including limited access to early detection services, timely diagnosis, and effective treatment. In LICs and LMICs, up to 60% of BC cases are diagnosed at Stage III or IV, compared to less than 30% in HICs, where organized screening programs have shifted diagnoses to earlier, more treatable stages (Fuentes et al., 2023).

By 2040, global BC cases are expected to rise to 3.19 million annually, with 1.04 million deaths, concentrated mostly in LICs and LMICs (The Lancet Oncology, 2024). Screening and early detection initiatives, which have been well-established in HICs over recent decades, have played a fundamental role in improving outcomes by facilitating the diagnosis of cancers at earlier, more treatable stages (WHO, 2010). These programs have also demonstrated the potential to significantly reduce the proportion of late-stage diagnoses, as seen in countries like Norway and Sweden (Fuentes et al., 2023). However, persistent barriers in LICs and LMICs, including lack of awareness, financial constraints, and cultural stigma, continue to hinder early detection efforts. These challenges contribute significantly to the disparities in outcomes observed between high- and low-income settings (Dey, 2014). These disparities underscore the importance of early detection strategies, mainly organized screening programs, which reduce mortality rates.

Figure 1: On the left, years lived with disability (YLD) per 100.000 in women aged 50-69 diagnosed with BC.

On the right, deaths per 100.000 in women aged 50-69 diagnosed with BC.



Screening and early detection

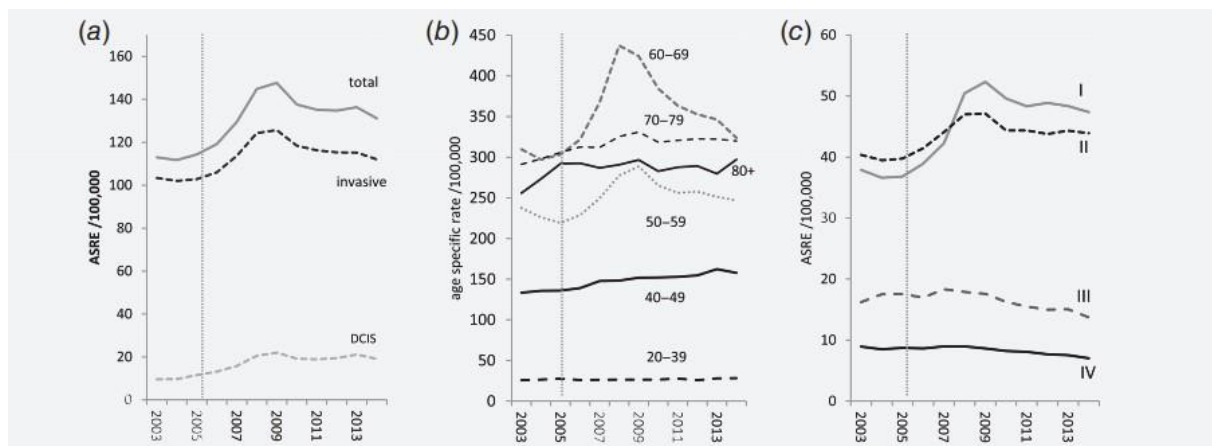
Screening for BC is an important secondary prevention strategy that has been shown to reduce mortality by facilitating early disease detection. BC screening can prevent BC morbidity and mortality by identifying cancer at an earlier stage than it would have presented clinically, improving survival rates (Trapani et al., 2023). Large clinical trials in North America and Europe have demonstrated that BC screening programs can save lives and increase longevity (Bretthauer et al., 2023). These programs are typically initiated through primary care providers or specialized screening programs.

In this context, it is important to distinguish between sporadic and organized BC screening programs. Organized BC screening programs systematically invite women in the target age group for regular mammography, ensuring consistent follow-up and equitable access (Lauby-Secretan et al., 2015; Gilbert & Pinker-Domenig, 2019). In contrast, sporadic or opportunistic screening relies on individuals or healthcare providers initiating screening, resulting in lower participation rates, gaps in follow-up, and a higher likelihood of late-stage detection (Lauby-Secretan et al., 2015). Evidence from structured programs demonstrates

their effectiveness in shifting the stage at which BC are diagnosed, with over 60% of invasive cancers detected at stage I or II in organized screening settings (Lousdal et al., 2016). This shift to earlier detection has been associated with significant reductions in mortality (Eby et al., 2022). Many European countries follow the guidelines established by the European Commission to implement Organized Screening Programs. These programs are designed to detect BC early in asymptomatic women by ensuring systematic invitations and high-quality, standardized mammography services (European Commission, 2022). Through adherence to these guidelines, European-organized screening programs have demonstrated notable reductions in BC mortality, demonstrating the effectiveness of structured and evidence-based approaches to early detection.

The implementation of organized BC screening programs has a well-documented initial impact on cancer incidence rates. This phenomenon is due to the detection of preclinical cancers that would otherwise remain undiagnosed for years. This phenomenon, often referred to as the "*incidence peak*," reflects the program's ability to identify cancers at earlier stages, including those that might not have become symptomatic within a woman's lifetime (Katalinic et al., 2019). The increase is especially pronounced in the first few rounds of screening as the program systematically includes women who may have never undergone mammography before. For instance, a European study found that organized screening programs increased BC incidence by approximately 16% within the first five years of implementation (Zielonke et al., 2020). The rise was attributed to the detection of early-stage and asymptomatic cancers. Following the initial spike in incidence, a stabilization or even a slight decline is observed over time as the population adjusts to regular screening intervals (Katalinic et al., 2019) (Figure 2).

Figure 2: Breast cancer incidence rates per 100,000 women in Germany, presented as follows: (a) age-standardized for all age groups, (b) stratified by age (invasive BC only), and (c) categorized by UICC stage (invasive BC only). The vertical dotted line marks the introduction of mammography screening in 2005. Adapted from Katalinic et al., 2019.



Meta-analyses of randomized clinical trials have further established the overall effectiveness of organized mammography screening, particularly for women aged 50 to 69, where it has been associated with a significant reduction in BC mortality. Biennial screening for women aged 50 to 69 provides a balance between maximizing the benefits of reduced mortality and minimizing the harms of false positives and overdiagnosis (Nelson et al., 2016). Current practice guidelines from professional societies (WHO), guideline groups, and government agencies recommend BC screening for average-risk women beginning no later than age 50 (WHO, 2023).

While the benefits of BC screening are well-documented, these programs can also bring some challenges. Overdiagnosis is a consequence of BC screening. An overdiagnosed BC refers to a cancer detected through screening that would not have caused symptoms or become clinically apparent during a woman's lifetime without screening (Gunsoy et al., 2012). BC screening tends to identify slow-growing cancers more often than fast-growing

tumours because slow-growing cancers remain in the detectable range of tumour sizes for a longer period, making them more likely to be picked up during screening (length bias) (Gøtzsche & Jørgensen, 2013). Overdiagnosis can result in unnecessary treatments, including surgery, radiation therapy, and chemotherapy, which bring physical, emotional, and financial burdens. Another concern is false positive results, in which screening detects abnormalities later found to be non-cancerous (Gøtzsche & Jørgensen, 2013). The false positives can cause considerable and sustained psychological distress also for years after the women are declared cancer-free. The cumulative risk of a false-positive result after 10 mammograms ranges from about 20% to 60% (Gøtzsche & Jørgensen, 2013). It was considerably higher in the US than elsewhere.

Socioeconomic inequalities in BC screening

Social and economic determinants of health (SDH) refer to the non-medical factors that significantly impact health outcomes. The WHO (2023) defines them as “*the conditions in which people are born, grow, work, live, and age*”. These determinants include economic systems and policies, social norms and policies, political systems, and developmental agendas. These factors influence health equity, which can be achieved by addressing and eliminating health inequities across populations defined by social, economic, demographic, or geographic characteristics (Li et al., 2024). Health inequalities are, according to the Commission on Social Determinants of Health, “*the result of the cumulative impact of decades of exposure to health risks of those who live in socioeconomically less advantaged circumstances*” (Marmot et al., 2008). Social inequalities in the cancer continuum refer to the systematic disparities among social groups that influence an individual’s risk of developing cancer, their access to timely and effective treatment, their chances of survival, and their availability to receive palliative care (Sarfati, 2019).) These social inequalities in the cancer continuum occur both within and between countries.

Evidence demonstrates the significant influence of socioeconomic inequalities on cancer incidence, survival after diagnosis, mortality at the population level, and access to screening (Tetzlaff et al., 2023). Studies have shown that individuals with lower socioeconomic positions, measured by education, income, employment status, or other indicators, are disproportionately at higher risk of being diagnosed with cancer and experiencing poorer outcomes, including higher mortality rates, compared to individuals in higher socioeconomic positions (Vaccarella et al., 2022). Moreover, particularly in HIC, cancer patients living in socioeconomically deprived areas are found to have worse cancer outcomes compared to those living in less deprived areas (Exarchakou et al., 2022).

Socioeconomic factors also significantly determine access to and uptake of BC screening programs. Evidence shows that women with lower socioeconomic status (SES), lower educational attainment, or lower income are less likely to participate in screening programs, as demonstrated in studies conducted in Canada, the UK, and Sweden (Mottram et al., 2021; Ding et al., 2022). Immigration status further exacerbates these disparities, with immigrants showing lower participation rates compared to native-born populations (Mottram et al., 2021). Evidence from a study conducted in the United States revealed that non-Hispanic Black women had slightly higher BC screening rates compared to non-Hispanic White women but experienced worse outcomes. In contrast, Hispanic and Asian women showed significantly lower screening rates, often due to systemic barriers such as language difficulties, lack of insurance, and reduced access to healthcare services (Sun et al., 2024). Structural barriers, such as distance from screening facilities and lack of transportation, disproportionately affect women in rural areas, as reported in studies from Australia and Norway, contributing to lower screening rates (Ding et al., 2022). Furthermore, health system factors, including reminders for screening, play a crucial role; reminders have been shown to increase participation, particularly in urban populations in the US and Sweden, where higher

non-participation rates were observed without reminders (Ding et al., 2022). Interestingly, cultural factors play a role in the uptake of BC screening, particularly in low-resource settings and among marginalized groups in higher-resource settings. In these settings, stigma surrounding BC and fear of diagnosis prevent women from participating in BC screening programs. Fatalistic beliefs in which cancer is perceived as an untreatable condition further discourage participation in screening programs. These barriers are often intensified by societal norms, where decision-making can be influenced by family members or community leaders (Tavakoli et al., 2024).

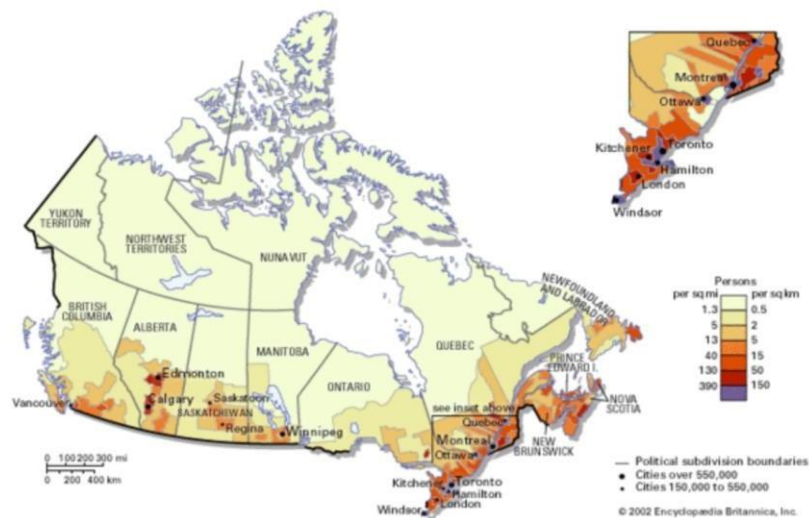
The need to address socioeconomic inequalities in cancer has gained particular attention in recent years, being recognized as a matter of human rights and economic benefit. Combating inequalities in cancer has been prioritized by the WHO and United Nations (UN) in the Public Health Agenda and in the Sustainable Development Goals (United Nations, 2015). Additionally, the European Union (EU) has made addressing inequalities in the cancer continuum a priority through initiatives such as the “Europe’s Beating Cancer Plan” and the establishment of the Cancer Inequalities Registry (European Commission, 2021).

The Canadian case study

Canada can be considered an interesting case study to understand differences and disparities in BC incidence and mortality, and screening participation inequalities. Canada is the second-largest country in the world by land area, composed of 10 provinces and three Territories. Provinces and Territories are responsible for social programs such as healthcare, education, and welfare. The difference between the Territories and the Provinces lies in their governance and administrative structure, where Provinces have more political power and autonomy than the Territories, which rely more on the Government of Canada. The vast majority of Canada is inhabited, with most of the population living close to the border with the

US and over 60% of the population living in two provinces: Quebec and Ontario. Compared with the Territories, which are located in the north, the southern regions of Canada are economically more affluent, with a high population density and high level of urbanization, and better-developed infrastructure and services, including healthcare facilities (Statistics Canada, 2022).

Figure 3: Canada population density map by Encyclopedia Britannica, 2021



Ontario stands out as a key province for investigating healthcare disparities. It is home to over 30% of Canada's population, with approximately 4.2 million immigrants and 406,590 Indigenous people, making it the province with the largest Indigenous population in Canada. Among the provinces, Ontario has the largest population and immigrant population in Canada, with the highest number of Indigenous residents, followed by British Columbia and Alberta. Despite having one of Canada's most developed healthcare infrastructures, Ontario still faces challenges in achieving equitable healthcare access, particularly for these minority populations (Martin et al., 2018).

Indigenous communities in Canada have historically faced social and economic inequities compared to the non-Indigenous population, stemming from the effects of colonization, systemic racism and forced displacement from their traditional land and communities (Durand-Moreau et al., 2022). These disparities have led to systemic challenges, including racism, discrimination, and bias within institutions, which still strongly impact the lives of Indigenous peoples (Durand-Moreau et al., 2022). The systemic disparities experienced by Indigenous populations in the Territories help explain the health inequities and socioeconomic disadvantages between them and the rest of Canada. Indigenous populations have a higher rate of type 2 diabetes, hypertension, substance abuse, and mental health problems (Durand-Moreau et al., 2022). Moreover, these populations are found to have a lower level of education and lower access to training compared to the non-Indigenous population, limiting their ability to get jobs in the mainstream economy (Durand-Moreau et al., 2022).

Similarly, systemic barriers also affect immigrant and visible minority groups, defined as individuals other than Indigenous who are non-Caucasian in race or non-white in colour (Table 2) (Statistics Canada, 2021). These face language and cultural barriers, as well as limited access to healthcare resources, exacerbating their marginalization within healthcare systems. These inequalities often stem from underlying inequities in the healthcare system, conditions that hinder access to reduced participation in preventive health measures, such as cancer screening programs. (Ferdous et al., 2018; Ahmed et al., 2015)

In this context, this study aims to analyze how socioeconomic factors influence breast cancer screening participation in the province of Ontario, focusing on four populations: Canadian-born, immigrant, Indigenous, and visible minorities. Specifically, this study aims to investigate differences in screening participation rates among these groups and investigate whether the socioeconomic factors influencing participation in BC screening differ across

these groups, interventions possibly highlighting unique barriers that each population faces and identifying possible areas for targeted intervention.

2.2 Material and Methods

Study design

This study conducted a secondary cross-sectional analysis using the 2019-2020 Canadian Community Health Survey (CCHS) dataset, focusing on the breast cancer screening module in Ontario

Data collection procedure

The CCHS is a cross-sectional survey conducted by Statistics Canada that collects information about health status, healthcare utilization and health determinants across Canada (Statistics Canada, 2019). CCHS facilitates population health research and allows for health surveillance. The 2019-2020 cycle of the CCHS, released in April 2022, was used for this analysis. Data were collected from approximately 110,000 respondents over the two-year period. The 2020 cycle had notable differences compared to the 2019 version due to the COVID-19 pandemic. Data collection was paused in March 2020 and did not resume until September, with collection periods shortened from three months to five weeks. In-person interviews were replaced by telephone interviews, which reduced the number of cases collected. In the territories, data collection was further limited during the final three collection periods of 2020, with coverage restricted to the capitals (Statistics Canada, 2022). However, the breast cancer screening data analyzed in this study was part of the core content administered in 2019 in Ontario, meaning that the disruptions caused by the COVID-19 pandemic did not affect the data of interest.

The CCHS employs a large sample to provide reliable estimates at national, provincial, and regional levels. The survey content is structured into annual common content, optional content, and rapid response content, each addressing different public health needs. Were conducted both in person and via phone call, lasting between 40 to 45 minutes (Statistics

Canada, 2022). The CCHS produces an annual microdata file and a second microdata file, which combine two years of data every second year. The combined data are the same as the two individual annual data files (Statistics Canada, 2022).

Research population and sampling

The 2019-2020 CCHS includes participants aged 18 years and older from all provinces and Territories of Canada. Individuals living on reserves and other Indigenous settlements, full-time members of the Canadian Forces, residents of certain remote regions (Quebec health regions of Région du Nunavik and Région des Terres-Cries-de-la-Baie-James), and institutionalized individuals were excluded from the data collection.

For the purpose of this study, female respondents aged 50 to 64 from the province of Ontario were included, as the BC screening module was only administered to women in that age range. Proxy reporting (when a selected respondent is unable to answer themselves) and cases with missing data were excluded from the analysis (Statistics Canada, 2022). In this study, the analysis was stratified into Canadian-born, immigrant, Indigenous peoples, and visible minorities to investigate specific barriers and facilitators of BC screening access within these groups. “Immigrant” were defined as being not born in Canada, and Indigenous peoples and visible minorities were defined based on the response given in the CCHS (Table 1).

Measures

This study utilized the Public Use Microdata Files (PUMF) retrieved from Statistics Canada (<https://www.statcan.gc.ca/eng/microdata/dli/data>). The BC screening questionnaire was part of the core content of the 2019-2020 edition. Participants were asked whether they had undergone a mammogram and, if so, the timing of their most recent test. The dependent variable, participation in BC screening, was defined as being up to date with recommended screening guidelines: a mammogram within the past two years for women aged 50 to 74, as per

the Ontario Breast Screening Program (OBSP) (Cancer Care Ontario, 2021). Other independent variables were included in the analysis to estimate the impact of socioeconomic and cultural factors on the uptake of BC screening. All variables included in the study are presented in Table 1:

Table 1: Variables included in the study, adapted from the CCHS data dictionary (Statistics Canada, 2022).

Variable Name		Associated Question
MAM_010	Up to date with mammography screening	When was the last time you had a mammography?
MAM_005	Mammography ever	Has the respondent ever had a mammogram?
SDC_015	Aboriginal status	Are you an Aboriginal person, that is, First Nations, Métis or Inuk (Inuit)?
SDCDVFLA	Visible minority	Do you belong to one or more racial or cultural groups?
PHC_020	Regular healthcare provider	Do you have a regular healthcare provider?
GENDVHDI	Perceived health	How do you consider your health to be?
INCDGHH	Income	What is the total household income?
EHG2DVH3	Education	What is the highest education level achieved by the respondent?
UCN_005	Unmet healthcare need	Did the respondent experience unmet healthcare needs?
CCCDGCAR		Does the respondent have any heart disease?
CCCDGSKL		Does the respondent have arthritis?
CCCDGRSP	Chronic conditions	Does the respondent have respiratory disease?
CCC_195		Does the respondent have high blood pressure?
CCC_200		Does the respondent have diabetes?
DHHGMS	Marital status	What is the respondent's marital status?
SMK_005	Smoking status	At the present time, do you smoke cigarettes every day, occasionally or not at all?
MAC_010	Working status last 12 months	Have you worked at a job or business at any time in the past 12 months?

Data analysis

The statistical analysis was carried out using SAS v.9.4 software. We calculated the proportion of participants who were up to date with BC screening. Descriptive statistics were stratified by Canadian-born, immigrant, Indigenous populations, and visible minorities to investigate differences in mammography screening status and the influence of socioeconomic factors. Descriptive statistics are presented as unweighted values and weighted percentages to improve population representativeness. Bivariate associations were used to investigate BC screening status by sociodemographic characteristics (including access to PHC). Multivariable logistic regression models were developed separately for each group to assess associations between socioeconomic factors and BC screening participation. Since the goal was to identify barriers within each subgroup and given the small sample sizes, separate logistic regression models were used instead of interaction terms in a pooled model. We used an α of less than 0.05 to determine statistical significance. Sample weights were applied in descriptive statistics, bivariate associations, and multivariable logistic regression. For each respondent, the sampling weight indicates the number of individuals in the Canadian population that the respondent represents (Statistics Canada, 2022). A thematic map was created using QGIS (v.3.x) to visualize the geographic distribution of BC screening prevalence, defined as women having had a mammography in the preceding 2 years across Ontario's public health units. Prevalence data were linked to the geographic shapefile of health unit boundaries using unique identifiers. A graduated symbology approach was applied, assigning a gradient colour ramp to represent screening prevalence, with lighter shades indicating lower prevalence and darker shades indicating higher prevalence.

Ethical considerations

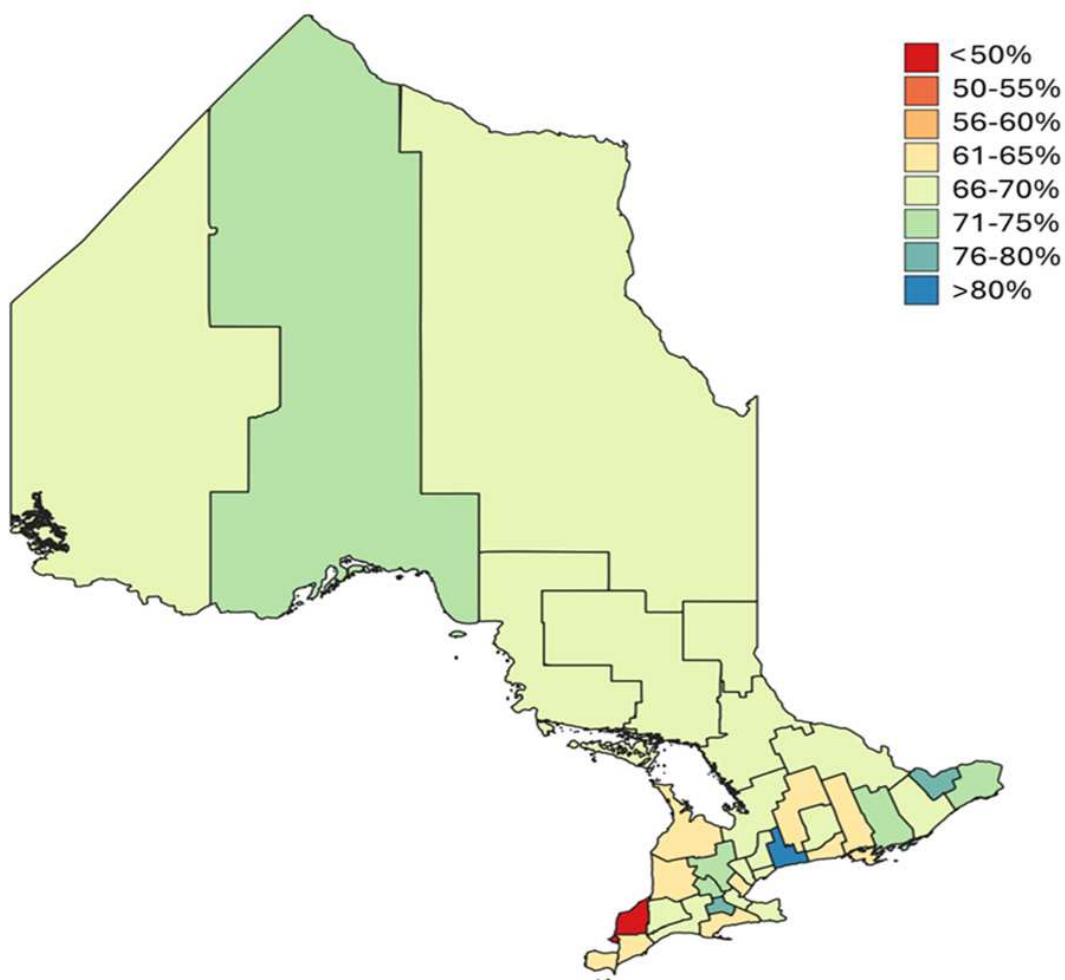
The CCHS dataset is collected by Statistics Canada per organizational data ethics principles. The CCHS PUMF data is accessed and adapted by the Statistics Canada Open

Licence Agreement (<https://www.statcan.gc.ca/en/reference/licence>). PUMFs are developed from the master files by removing or collapsing variables that could potentially lead to the identification of individuals. Variables most likely to identify an individual are deleted or collapsed into broader categories (Statistics Canada, 2022).

2.3 Results

A thematic map (Figure 3) illustrates the geographic distribution of BC screening uptake across Public Health Units (PHUs) in Ontario, revealing notable regional disparities. Urban PHUs generally exhibit higher screening uptake compared to rural and remote areas. Notably, the Durham Region Health Department and Brant County Health Unit report BC screening rates exceeding 75%, at 80.5% and 78.8%, respectively. In contrast, Lambton Public Health demonstrates a significantly lower prevalence, with only 47% of individuals up to date with screening.

Figure 4: Adherence to BC screening per Public Health Unit (LHU) in Ontario in women aged 50-64, 2019



The study included 3,937 female respondents, subdivided by Canadian-born (3,097), immigrant (747), Indigenous (116), and visible minority populations (370) (Table 2).

Table 2: Definitions and sample sizes of study populations stratified by Canadian-born, immigrant, Indigenous, and visible minority groups. The table describes the inclusion criteria and respective sample sizes for women aged 50-64 residing in Ontario

Name	Definition	Sample size
Canadian-born	Women aged 50-64 resident in Ontario born in Canada	3097
Immigrant	Women aged 50-64 resident in Ontario not born in Canada	747
Indigenous	Women aged 50-64 resident in Ontario who identify as Indigenous (First Nation, Inuit or Métis)	116
Visible minorities	Women aged 50-64 resident in Ontario that belong to one or more racial/cultural groups**	370

** Statistics Canada (2021) defines visible minorities as “persons, other than Indigenous peoples, who are non-Caucasian in race or non-white in colour”. The visible minority population consists mainly of the following groups: South Asian, Chinese, Black, Filipino, Arab, Latin American, Southeast Asian, West Asian, Korean and Japanese.”

Table 3 presents the lifetime mammography rates and uptake of BC cancer screening across these groups. Among the Canadian-born population, 91.4% reported ever having a mammogram, compared to 90.1% in the immigrant population, 94.6% among Indigenous individuals, and 85.7% among visible minorities. Notably, Indigenous respondents exhibited the highest rate of lifetime mammography participation, while visible minorities had the lowest. Among the general population, 69.1% of women were up-to-date with BC screening, compared to where this proportion was 68.8% in the Canadian-born population, 70.6% in the immigrant population, and 67.0% in the Indigenous population. Immigrant respondents exhibited the highest rate of regular participation in BC screening, while Indigenous respondents had the lowest. Similarly, 67.5% of visible minorities were up-to-date with BC screening (Table 3)

Table 3: Lifetime and up-to-date mammography screening participation rates among Canadian-born, immigrant, and Indigenous, presenting proportions of "Yes," age group 60-64

	General population	Canadian-born population	Immigrant population	Indigenous population	Visible minority
	%	%	%	%	%
Lifetime mammography Yes (%)	90.5	91.4	90.1	94.6	85.7
Up-to-date screening Yes (%)	69.1	68.8	70.6	67.0	67.5

*Note: % (percentage) of women who had mammography in their lifetime and women who are up to date with BC screening. Percentages are presented as weighted

Table 4 presents the socioeconomic characteristics of respondents. Most respondents in all groups reported access to a regular healthcare provider, with the highest proportion among visible minorities (97.9%) and the lowest among immigrants (97.3%). Compared to the general population, both immigrants ($p = 0.01$) and visible minorities ($p = 0.01$) were significantly more likely to have access to a regular healthcare provider. Education level was highest among visible minorities, with 85.2% holding a post-secondary diploma or certificate, compared to 64.8% in Indigenous populations. The difference was statistically significant for visible minorities ($p = 0.001$), who had a higher proportion of post-secondary graduates than the general population. Secondary school graduation rates were significantly lower among immigrants (10.4%, $p = 0.0009$), Canadian-born respondents (13.3%, $p = 0.0003$), and visible minorities (8.2%, $p = 0.001$) compared to the general population. Household income varied significantly across groups. Indigenous respondents were significantly less likely to report earning over \$60,000 (62.8%, $p = 0.009$) compared to the general population. Marriage rates were highest among immigrants (75.9%) and visible minorities (72.8%), though no statistically significant differences were observed compared to the general population

Perceived health was highest among Canadian-born respondents, with 38.5% reporting 'very good' health and 22.1% reporting 'excellent' health, while Indigenous and visible minority respondents reported lower perceived health levels. Compared to the general population, immigrants ($p = 0.0001$), Indigenous respondents ($p = 0.02$), and visible minorities ($p = 0.001$) were significantly more likely to report poorer perceived health. Chronic conditions were most prevalent among Indigenous respondents (59.6%) and least prevalent among visible minorities (33.8%). Compared to the general population, Indigenous respondents had a significantly higher prevalence of chronic conditions ($p = 0.004$), while visible minorities had a significantly lower prevalence ($p = 0.001$). Smoking rates were lowest among visible minorities (3.9%) and immigrants (5.0%) compared to the general population, with statistically significant differences observed for both groups ($p = 0.001$). Conversely, Indigenous respondents (13.9%) had higher smoking rates, though this difference was not statistically significant ($p = 0.51$). Employment rates were highest among immigrants (73.9%) and visible minorities (74.8%). Both groups were significantly more likely to be employed compared to the general population ($p = 0.001$). Unmet healthcare needs were reported most frequently by Indigenous respondents (13.3%), a significantly higher proportion compared to the general population ($p = 0.005$). In contrast, visible minorities had the lowest proportion (6.7%), though this difference was not statistically significant ($p = 0.09$).

Table 4: Socioeconomic characteristics and health status of women aged 50–64 stratified by Canadian-born, immigrant, Indigenous, non-visible minority, and visible minority groups.

	General population	Canadian-born population		Immigrant population		Indigenous population		Visible minority	
	N (%)	N (%)	p-value	N (%)	p-value	N (%)	p-value	N (%)	p-value
Access to a regular healthcare provider									
Yes	222 (95.8)	2906 (95.6)	0.07	719 (97.3)	0.01	103 (96.0)	0.86	361 (97.9)	0.01
No	3701 (3.8)	187 (4.2)		28 (2.7)		13 (4.0)		9 (2.1)	
Education level									
Less than secondary school graduation	90 (2.3)	63 (1.2)	0.0003	22 (2.3)	0.0009	9 (2.7)	0.18	16 (2.9)	0.001
Secondary school graduation	661 (16.8)	552 (13.3)		88 (10.4)		10 (5.1)		35 (8.2)	
Post-secondary certificate/diploma /	2941 (74.7)	2282 (79.8)		599 (83.5)		69 (64.8)		302 (85.2)	
Household income									
less than \$20000 CAD	272 (6.9)	223 (3.45)	0.009	40 (2.5)	0.12	21 (7.24)	0.009	22 (2.4)	0.41
\$20000 - \$59999	1075 (28.8)	803 (19.0)		211 (21.3)		36 (27.8)		99 (20.0)	
more than \$60000	2511 (63.8)	1998 (76.7)		477 (75.72)		54 (62.8)		246 (77.4)	
Marriage									
Yes	2292 (58.2)	1791 (72.7)	0.44	446 (75.9)	0.06	59 (69.0)	0.39	212 (72.8)	0.45
No	1624 (41.6)	1290 (27.0)		297 (23.8)		56 (30.7)		157 (26.9)	

Table 4: Socioeconomic characteristics and health status of women aged 50–64 stratified by Canadian-born, immigrant, Indigenous, and visible minority groups.

	General population	Canadian-born population		Immigrant population		Indigenous population		Visible minority	
	N (%)	N (%)	p-value	N (%)	p-value	N (%)	p-value	N (%)	p-value
Perceived health									
Poor	199 (3.9)	159 (3.5)		33 (4.4)		14 (3.39)		15 (4.5)	
Fair	462 (9.7)	368 (10.5)		74 (7.9)		24 (18.0)		38 (7.0)	
Good	1127 (29.0)	851 (25.3)	0.0001	248 (36.6)	0.0001	34 (26.3)	0.02	126 (34.3)	0.001
Very good	1352 (36.7)	1084 (38.5)		241 (32.3)		29 (25.9)		120 (36.1)	
Excellent	792 (20.7)	632 (22.1)		149 (18.8)		15 (26.4)		70 (18.0)	
Chronic condition									
Yes	2011 (49.0)	1653 (52.7)		392 (38.0)		70 (59.6)		146 (33.8)	
No	1700 (47.4)	1273 (44.0)	0.0001	304 (58.6)	0.001	31 (31.9)	0.004	208 (61.7)	0.001
Smoking status									
Yes	607 (12.6)	541 (16.1)		51 (5.0)		29 (13.9)		12 (3.9)	
No	3194 (85.7)	2444 (70.3)	0.0001	667 (94.1)	0.001	70 (79.6)	0.51	351 (95.1)	0.001
Working status									
Yes	2640	2055 (70.3)		532 (73.9)		69 (68.0)		259 (74.8)	
No	1259 (27.4)	1021 (29.2)	0.01	201 (24.3)	0.001	47 (32.0)	0.28	103 (23.1)	0.001
Unmet needs health									
Yes	228 (5.4)	186 (5.3)	0.12	39 (5.9)		12 (13.3)		20 (6.7)	
No	3683 (93.9)	2908 (94.6)		706 (93.9)	0.40	102 (86.4)	0.005	350 (93.3)	0.09

*Note: The general population was used as the reference category; N (number) was presented as unweighted, and % (percentage) was presented as weighted. Missing responses: Access to a regular healthcare provider (14), Education level (245), Household income (97), Marriage (21), Perceived health (5), Chronic condition (226), Smoking status (136), Working status (94), Unmet healthcare needs (26). Percentages exclude missing values unless stated otherwise.

The results presented in Table 5 show the bivariate associations between socioeconomic factors and BC screening status across the populations of interest. Chronic conditions were significantly associated with BC screening among visible minorities ($p = 0.01$). Access to a regular healthcare provider was strongly associated with screening in Canadian-born populations ($p < 0.001$). Education level was significantly associated with screening uptake in the immigrant population ($p = 0.0002$). Marital status was significantly associated with BC screening in Canadian-born populations ($p = 0.01$). Perceived health was a predictor of screening participation among Canadian-born ($p = 0.02$), immigrant ($p = 0.037$), and visible minority populations ($p < 0.0001$). Household income was significantly associated with screening among visible minorities ($p = 0.013$). Smoking status emerged as a significant factor across Canadian-born ($p = 0.0007$), immigrant ($p < 0.0001$), Indigenous ($p < 0.0001$), and visible minority populations ($p < 0.0001$). Working status was significantly associated with screening participation in immigrant ($p < 0.0001$) and Indigenous populations ($p < 0.0001$). Unmet healthcare needs significantly influenced screening among immigrant ($p = 0.0003$) and visible minority populations ($p < 0.0001$).

Table 5: Bivariate associations between socioeconomic determinants and mammography screening status among women aged 50–64, stratified by Canadian-born, immigrant, Indigenous, and visible minority groups.

		Canadian-born population			Immigrant population			Indigenous population			Visible minorities		
		Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value
Chronic conditions	Yes	74.5	25.5	0.11	75.8	24.2	0.09	80.7	19.2	0.01	80.4	19.6	0.22
	No	74.5	25.5		79.9	20.1		53.5	46.5		76.4	23.6	
Access to a regular healthcare provider	Yes	76.9	23.1	<0.001	78.6	21.4	0.51	72.3	27.7	0.17	78.8	21.2	0.82
	No	51.3	48.7		83.9	16.1		37	63		75.7	24.3	
Education level	Less than secondary school graduation	58.5	41.5	0.1	93.3	6.7	0.0002	81.4	18.5	0.85	100	0	0.06
	Secondary school graduation	77.5	22.5		65.6	34.4		63.5	36.5		79.8	20.2	
	Post-secondary certificate/diploma	76.6	23.4		80.5	19.5		74.6	25.4		78.4	21.6	
Marital status	Yes	77.4	22.6	0.01	78.7	21.3	0.99	74.1	25.9	0.33	77.5	22.5	0.21
	No	72.3	27.7		78.6	23.4		63.3	36.7		81.9	18.1	

Table 5: Bivariate associations between socioeconomic determinants and mammography screening status among women aged 50–64, stratified by Canadian-born, immigrant, Indigenous, and visible minority groups.

		Canadian born population			Immigrant population			Indigenous population			Visible minorities		
		Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value	Up to date with BCs (%)	Not up to date with BCs (%)	p-value
Self-perceived health	Poor	62.2	37.8		86.9	13.1		47.1	52.9		91.4	8.6	
	Fair	71.7	28.3	0.02	81.5	18.5	0.037	76.7	23.3	0.92	92.8	7.1	<.0001
	Good	77.9	22.1		77.3	22.7		71.1	28.9		76.6	23.4	
	Very good	76.5	23.5		82	18		72.4	27.6		84.6	15.4	
	Excelent	77.1	22.8		72.8	27.2		67	33		64.8	35.2	
Household income	less than \$20000 CAD	71.5	28.5		71.8	28.2		48.6	51.4		80.8	19.2	
	\$20000 - \$59999	77.3	22.7	0.57	72.4	27.6	0.013	95.4	4.6	0.01	75	25	0.49
	more than \$60000	76.2	23.8		80.6	19.4		62.5	37.5		79.6	20.4	
Smoking	Yes	68.3	31.7	0.0007	56.6	43.4	<.0001	64.6	35.4	0.0001	27.6	72.4	<.0001
Working	Yes	76.2	23.8		75.4	24.6		68.3	31.7		74.6	25.4	
	No	75.5	24.5	0.73	87.9	12.1	<.0001	76.2	23.8	0.47	91.2	8.8	<.0001
Unmet needs of health	Yes	77.9	22	0.62	62.1	37.9	0.0003	83.2	16.8	0.37	54.7	45.3	<.0001
	No	75.9	24		79.8	20.2		69.2	30.8		80.8	19.2	

Note: BCs: breast cancer screening. Proportions (%) represent the percentage of individuals within each category (up-to-date or not up-to-date with breast cancer screening) for the respective variables and population

Tables 6a and 6b present crude and adjusted OR for each population of interest. Based on the preliminary analysis findings, logistic regression models were constructed. Due to the small sample sizes for the Indigenous population and visible minorities, only variables with the strongest associations or those deemed most relevant based on prior research were included in the model to maintain statistical power. Multicollinearity among independent variables was assessed using the Variance Inflation Factor (VIF). All VIF values were below the commonly accepted threshold of 5, indicating no significant multicollinearity issues.

Across all population groups, some factors consistently influenced BC screening participation, while others varied significantly. Among Canadian-born women, having a regular healthcare provider had a strong positive association (adjusted OR = 3.26, 95% CI: 2.00–5.31, $p < 0.0001$). Similarly, being married (adjusted OR = 1.36, 95% CI: 1.04–1.78, $p = 0.02$) and better self-perceived health (e.g., “excellent” health: adjusted OR = 2.6, 95% CI: 1.43–4.72, $p = 0.002$) were positively linked to screening uptake in this group. Smoking was associated with lower BC screening participation (adjusted OR = 0.61, 95% CI: 0.46–0.82, $p = 0.001$). Among the immigrant population, chronic conditions significantly reduced screening participation (adjusted OR = 0.55, 95% CI: 0.33–0.93, $p = 0.03$), while education and income showed non-significant positive trends (e.g., income > \$60,000: adjusted OR = 1.95, 95% CI: 0.43–8.74, $p = 0.39$). Smoking also showed a negative effect on BC screening uptake (adjusted OR = 0.64, 95% CI: 0.28–1.47, $p = 0.3$), though it was not statistically significant. For Indigenous women, chronic conditions were positively associated with screening (adjusted OR = 3.68, 95% CI: 1.13–11.97, $p = 0.03$), and access to a regular healthcare provider showed a positive, though non-significant, trend (adjusted OR = 4.23, 95% CI: 0.20–88.65, $p = 0.35$). Smoking did not significantly affect BC screening participation in this group (adjusted OR = 1.12, 95% CI: 0.15–8.65, $p = 0.91$). Among visible minorities, smoking (adjusted OR = 0.09, 95% CI: 0.02–0.38, $p = 0.01$) and unmet healthcare

needs (adjusted OR = 0.25, 95% CI: 0.12–0.55, $p = 0.01$) were significant barriers to being up to date with BC screening. Unexpectedly, better-perceived health in this group was associated with reduced odds of screening uptake (e.g., “excellent” health: adjusted OR = 0.15, 95% CI: 0.02–1.06, $p = 0.02$)

Table 6a: Crude and adjusted odds ratios (OR) for having mammography within the past 2 years among women aged 50–64, with key socioeconomic determinants, stratified by Canadian-born and immigrant populations

		Canadian-born population						Immigrant population					
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Access to a regular healthcare provider	Yes	3.176	(2.01, 5.03)	<0.0001	3.26	(2.00, 5.31)	<0.0001	0.704	(0.24; 2.03)	0.516	0.34	(0.06, 1.97)	0.23
	No	ref											
Education level	Secondary school graduation	2.43	(1.03, 5.75)	0.04	1.87	(0.72, 4.84)	0.2	0.13	(0.029; 0.65)	0.012	0.32	(0.04, 2.24)	0.15
	Post-secondary education	2.32	(1.02, 5.27)	0.04	1.57	(0.62, 3.93)	0.34	0.29	(0.06; 1.36)	1.36	1.62	(0.22, 11.76)	0.89
	Less than secondary school graduation	ref											
Income level	\$20,000-\$59,999	1.35	(0.76, 2.41)	0.29	1.16	(0.60, 2.24)	0.66	1.03	(0.44; 2.40)	0.94	1.04	(0.21, 4.72)	0.9
	>\$60,000	1.27	(0.74, 2.17)	0.38	0.86	(0.45, 1.65)	0.65	1.63	(0.72; 3.68)	0.24	1.95	(0.43, 8.74)	0.39
	<\$20,000	ref											
Marriage status	Yes	1.31	(1.05, 1.63)	0.01	1.36	(1.04, 1.78)	0.02	1.01	(0.72; 1.38)	0.99	0.96	(0.48, 1.89)	0.91
	No	ref											

Table 6a: Crude and adjusted odds ratios (OR) for having mammography within the past 2 years among women aged 50–64, with key socioeconomic determinants, stratified by Canadian-born and immigrant populations

		Canadian-born population						Immigrant population					
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Perceived health	Fair	1.54	(0.88; 2.68)	0.12	1.9	(1.02, 3.53)	0.04	0.66	(0.26; 1.69)	0.396	0.52	(0.10, 2.63)	0.07
	Good	2.13	(1.28; 3.56)	0.003	2.6	(1.48, 4.59)	<0.0001	0.51	(0.22; 1.15)	0.106	0.13	(0.06, 1.09)	0.18
	Very good	1.97	(1.20, 3.24)	0.007	2.3	(1.30, 4.02)	0.003	0.69	(0.30; 1.57)	0.378	0.31	(0.07, 1.38)	0.12
	Excellent	2.04	(1.23, 3.42)	0.006	2.6	(1.43, 4.72)	0.002	0.4	(0.17; 0.93)	0.033	0.34	(0.07, 1.64)	0.18
	Poor	ref											
Chronic condition	Yes	1.17	(0.96, 1.43)	0.11	1.21	(0.96, 1.53)	0.1	0.78	(0.59; 1.04)	0.1	0.55	(0.33, 0.93)	0.03
	No	ref											
Smoking status	Yes	0.63	(0.48, 0.82)	0.0007	0.61	(0.46, 0.82)	0.001	0.32	(0.18; 0.55)	<0.001	0.64	(0.28, 1.47)	0.3
	No	ref											
Working status	Yes	1.04	(0.83; 1.28)	0.73	1.04	(0.83, 1.28)	0.73	0.42	(0.28; 0.62)	<0.001	0.54	(0.27, 1.06)	0.08
	No	ref											
Unmet healthcare needs	Yes	1.12	(0.70, 1.78)	0.63	1.22	(0.73, 2.02)	0.45	0.41	(0.25; 0.67)	<0.001	0.76	(0.22, 2.57)	0.66
	No	ref											

Table 6b: Crude and adjusted odds ratios (OR) for having mammography within the past 2 years among women aged 50–64, with key socioeconomic determinants, stratified by Canadian-born and Indigenous populations

		Indigenous population						Visible minorities					
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Access to a regular healthcare provider	Yes	4.44	(0.43; 45.57)	0.2	4.23	(0.20, 88.65)	0.35	1.195	(0.23; 6.07)	0.8	1.41	(0.25, 7.84)	0.07
	No	ref											
Education level	Secondary school graduation	0.39	(0.01; 19.54)	0.64									
	Post-secondary education	0.66	(0.02; 20.43)	0.81									
	Less than secondary school graduation	ref											
Income level	\$20,000-\$59,999	22.1	(1.57; 377.74)	0.02				0.71	(0.19; 2.54)	0.6	2.94	(0.64, 13.45)	0.16
	>\$60,000	1.76	(0.29; 10.41)	0.53				0.92	(0.27; 3.16)	0.9	4.25	(0.96, 18.89)	0.06
	<\$20,000	ref											
Marriage status	Yes	0.61	(0.21; 1.70)	0.34				0.76	(0.49; 1.16)	0.21	0.58	(0.34, 0.98)	0.04
	No	ref											

Table 6b: Crude and adjusted odds ratios (OR) for having mammography within the past 2 years among women aged 50–64, with key socioeconomic determinants, stratified by Canadian-born and Indigenous populations

		Indigenous population						Visible minorities					
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Perceived health	Fair	3.7	(0.15; 87.42)	0.41				1.22	(0.24; 5.97)	0.806	0.79	(0.09, 7.39)	0.39
	Good	2.77	(1.26;61.01)	0.51				0.31	(0.09; 1.026)	0.055	0.24	(0.04, 1.70)	0.12
	Very good	2.94	(0.13; 64.21)	0.49				0.51	(0.15; 1.78)	0.289	0.45	(0.06, 3.24)	0.12
	Excellent	2.28	(0.10; 49.31)	0.59				0.17	(0.05; 0.58)	0.004	0.15	(0.02, 1.06)	0.02
	Poor	ref											
Chronic condition	Yes	3.64	(1.27; 10.46)	0.01	3.68	(1.13, 11.97)	0.03	1.29	(0.87; 1.85)	0.22	1.31	(0.80, 2.13)	0.21
	No	ref											
Smoking status	Yes	0.73	(0.18; 2.96)	0.66	1.12	(0.15, 8.65)	0.91	0.28	(0.16; 0.50)	<0.001	0.09	(0.02, 0.38)	0.01
	No	ref											
Working status	Yes	0.67	(0.23; 1.99)	0.47				0.092	(0.03; 0.27)	<0.001	0.28	(0.14, 0.58)	0.02
	No	ref											
Unmet healthcare needs	Yes	2.2	(0.37; 12.90)	0.38	15.4	(0.11, >999.9)	0.28	0.28	(0.16; 0.50)	<0.001	0.25	(0.12, 0.55)	0.01
	No	ref											

* Adjusted odds ratios (ORs) were calculated for the following covariates: access to a regular healthcare provider, income, marital status, perceived health, chronic conditions, smoking status, working status, and unmet healthcare needs. For Aboriginal and visible minorities, models excluded certain variables (e.g., education for visible minorities; education, income, marital status, perceived health, and working status for Aboriginals) due to sample size constraints.

2.4 Discussion

In this study, we aimed to assess the influence of socioeconomic factors linked to regular access to BC screening services in Ontario. The study focused on four population subgroups: Canadian-born, immigrant, Indigenous, and visible minority, with the aim of identifying associations between socioeconomic factors and regular access to BC screening services and participation across these groups.

Our findings revealed differences in socioeconomic factors influencing BC screening participation across the four above-mentioned populations and geographic regions. Screening rates were similar across the population investigated, with immigrant women exhibiting the highest rates of being up-to-date with BC screening (70.6%), while Indigenous women had the lowest rates (67.0%). Geographically, urban areas such as Durham (80.5%) and Brant County (78.8%) demonstrated higher screening participation than rural regions like Lambton (47%). These data suggest that geographic location might play a prominent role in influencing regular access to BC screening compared to other factors, such as cultural or ethnic considerations. However, further investigation would be needed to confirm this.

Furthermore, our analysis revealed differences in the socioeconomic factors influencing BC screening among the above-mentioned population. Among Canadian-born women, access to regular healthcare providers, marital status and perceived health were positively associated with BC screening participation; meanwhile, smoking was found to be a significant barrier. Immigrant women with post-secondary education and higher income showed higher odds of being up to date with BC screening. Interestingly, access to a regular healthcare provider negatively affected screening participation. In the Indigenous populations, chronic conditions were found to be a strong correlate of regular involvement in BC screening. Moreover, access to a regular healthcare provider demonstrated a positive

association with BC screening participation. Notably, among visible minorities, smoking, unmet healthcare needs and working status were negatively associated with regular BC screening participation as well as better perceived health.

Data on BC screening uptake in Ontario align with those found in literature and policy reports, with the Ontario Cancer Screening Performance Report reporting rates between 61% and 68% between 2000 and 2018 (Cancer Care Ontario, 2020). Our study demonstrates regular access to BC screening, with 69.1% of the general population screened regularly. Interestingly, this study found high screening rates among immigrant women. An explanation for this can be found in the healthy immigrant bias, which suggests that economic immigrants tend to arrive in better health and might adopt healthier behaviours more easily compared to the Canadian-born population (Brabete, 2017). This phenomenon is mainly attributed to selective immigration policies and self-selection, where individuals who migrate are typically healthier and more resourceful (McDonald & Kennedy, 2004). However, the healthy immigrant effect tends to diminish with longer residency in the host country, often due to exposure to socioeconomic challenges and acculturation. In some cases, the decline in health advantages among immigrants results in their health status aligning with that of the Canadian-born population. In other situations, the health of immigrants deteriorates beyond that of the Canadian-born population (overshoot) (Vang et al., 2015).

Geographical differences in BC screening reported in this analysis also align with the literature, with a study carried out in Canada highlighting those individuals living in smaller urban areas or rural regions had lower odds of not participating in BC screening compared to those living in larger metropolitan areas (Gold et al., 2023). This trend was consistent in other HICs in which screening rates were decreased in rural residents compared to urban residents (Walji et al., 2021).

The influence of socioeconomic factors on BC screening participation varied significantly across the populations under study. For Canadian-born women, having access to a regular healthcare provider was a critical factor in the regular uptake of BC screening, consistent with other studies highlighting the importance of primary healthcare access in facilitating cancer preventive care utilization (Emery et al., 2013). In particular, the survey carried out by Prowse et al. (2024) highlighted that regular contact with healthcare providers increases access to screening services, encouraging adherence to preventive care recommendations. In addition to healthcare access, marital status was positively associated with regular participation in BC screening in Canadian-born women. These results align with the literature, demonstrating that marriage may be a proxy for social support, enhancing health-seeking behaviours and increasing access to preventive care (Hanske et al., 2016). Better perceived health was also positively associated with regular BC screening, possibly reflecting an increased awareness. Conversely, smoking was found to be a significant barrier to regular BC screening in Canadian-born women, consistent with data showing reduced engagement in preventative health behaviours compared to never smoking (Sanford et al., 2019).

Higher income levels and higher educational attainment were positively associated with regular BC screening participation in the immigrant population. These findings align with existing literature highlighting the role of income and education level in accessing cancer prevention screening, particularly among immigrants (Ferdous et al., 2019). However, access to regular healthcare providers appears to have a negative, though insignificant, association with BC screening participation in this group. Interestingly, this result does not align with the literature, which shows that regular access to healthcare providers is linked to increased access to cancer prevention programs (Prowse et al., 2024).

Among Indigenous women, when adjusted for other variables, chronic conditions were found to be a significant correlate of BC screening participation, possibly suggesting that individuals diagnosed with a chronic illness might be more engaged and knowledgeable about the healthcare system compared with Indigenous women without chronic illness, facilitating preventive screening uptake. Tarasenko et al. (2013) highlighted that individuals managing chronic conditions often have more significant contact with healthcare providers, which may increase opportunities for preventive care recommendations. While access to a regular healthcare provider showed a positive trend, this association was not statistically significant, possibly reflecting the Indigenous population's systemic barriers, including geographic barriers and mistrust of healthcare services (Durand-Moreau et al., 2022). These barriers, combined with the limited availability of culturally appropriate healthcare services, may contribute to the lower screening rates observed among Indigenous women. Additionally, this result could be attributed to the small sample size, which may limit the statistical power to detect significant associations.

Visible minorities showed distinct barriers to regular BC screening participation. Smoking, unmet healthcare needs, and working status were negatively associated with regular BC screening participation. These findings highlight the structural inequities and priorities that affect this group. Moreover, the negative association between better-perceived health and screening participation differs from the trends observed in other populations. This finding may reflect cultural beliefs or systemic factors that discourage preventive care, even among those who perceive themselves as healthy. Similar trends have been reported in studies on healthcare mistrust and stigma among minority populations (Kasper et al., 2024). Similarly, this result could be attributed to the small sample size, which limits the statistical power to detect significant associations.

This study highlights the role of socioeconomic factors in BC screening within different subgroups. The results emphasize that each population experiences unique barriers and facilitators. For instance, access to a regular healthcare provider was a critical BC screening facilitator for Canadian-born and Indigenous women. However, this factor showed weaker or negative associations among immigrant and visible minority women.

This study highlights the importance of addressing socioeconomic, structural, and systemic barriers to ensure equitable access to breast cancer screening services across diverse populations in Ontario. Culturally and linguistically tailored outreach programs, community engagement, and digital tools could improve participation for visible minority populations. Indigenous women, facing unique challenges like geographic isolation and systemic barriers, may benefit from mobile screening units, telehealth services, and the involvement of Indigenous healthcare workers to enhance trust and accessibility. Workplace policies supporting preventive health, such as paid leave for medical appointments, are crucial for immigrant and visible minority women. Collaborative approaches involving target communities in designing interventions are essential to ensure strategies effectively address their needs and preferences, ultimately supporting increased screening uptake and reducing inequities (Richardson-Parry et al., 2023; Ahmed & Shahid, 2012).

Strengths and limitations

Several strengths can be found in this study. Firstly, this study presents the analysis subdivided across different populations, allowing the investigation of specific barriers and facilitators for each population. Another strength is using the CCHS as a dataset to investigate regular BC screening uptake, as it ensures a robust sample size and population level representativeness. Moreover, the analysis includes different socioeconomic variables, such as education, income, perceived health, smoking status and access to healthcare. The

analysis also uses stratified multivariable models, allowing the identification of barriers and facilitators in each population investigated.

As for limitations, this study uses a cross-sectional dataset, which can limit the ability to infer causality about the relationship between socioeconomic factors and BC screening uptake. Additionally, the CCHS excluded individuals living on reserves, in remote regions, and other institutionalized populations, which may have led to the underrepresentation of some Indigenous and marginalized groups. While the age range for BC screening is typically 50 to 74 years, this analysis was limited to women aged 50 to 69 because of the design of the CCHS questionnaire, which only included relevant questions for this age group. Furthermore, the small sample size for Indigenous women (116 respondents) limits the statistical power of the analysis and may affect the generalizability of the findings for this population subgroup. A limitation of this study is the treatment of groups, Canadian-born, immigrants, First Nations, and visible minorities, as stratified and mutually exclusive despite individuals potentially belonging to multiple categories. This simplification may overlook the compounded effects of overlapping identities. However, this approach was necessary to focus on the barriers and facilitators experienced by each group, allowing for the analysis and targeted recommendations. While this limits the exploration of intersectional dynamics, it provides insights within the constraints of the available dataset.

Conclusions

In conclusion, this study highlights the influence of covariation between socioeconomic factors on BC screening participation in Ontario, demonstrating that these factors vary among Canadian-born, immigrant, Indigenous, and visible minority women. By identifying population-specific barriers and facilitators, this study underscores the importance

of tailored public health strategies to address disparities in screening uptake and improve equity in cancer prevention.

Conclusion: The role of socioeconomic determinants in health outcomes

This thesis has explored the impact of SDH on two distinct areas: healthy aging in Italy and disparities in breast cancer screening participation in Canada. While these topics may appear different, they share a common topic: the recognition that health outcomes are shaped not only by biological factors but also by the broader social, economic, and structural conditions in which individuals live.

The Novara Cohort Study (NCS) and its pilot study illustrate how socioeconomic status, education, and lifestyle factors can influence aging trajectories and highlight the need to investigate these factors. The study underscores the importance of integrating social and economic variables into aging studies, acknowledging that the accumulation of disadvantages over a lifetime contributes to disparities in health and well-being in later years.

Similarly, the analysis of breast cancer screening participation in Ontario highlights how SDH create barriers to preventive healthcare, particularly for Indigenous and immigrant populations. The study demonstrates that healthcare access, income, education, and structural inequities significantly affect participation in screening programs, leading to disparities in early detection and cancer outcomes. These findings reinforce the notion that health is not just an individual attribute but a product of systemic factors that influence healthcare access and utilization.

Together, these studies contribute to the growing evidence that health disparities are deeply rooted in social structures. Addressing these inequities requires a shift from individualized approaches to more comprehensive public health strategies that recognize and mitigate the impact of social determinants. Policies to reduce health disparities must go beyond healthcare provision alone, encompassing broader social policies that address economic inequality, education, housing, and community infrastructure.

The insights gained from this research highlight the critical need to integrate SDH into public health initiatives, epidemiological research, and health policy planning. Whether in the context of aging or cancer prevention, achieving health equity necessitates an approach that ensures all individuals, regardless of their socioeconomic background, can live healthier lives. By recognizing the interplay between social, economic, and health factors, we can move towards a more inclusive and equitable healthcare system, prioritizing prevention, accessibility, and long-term well-being for all.



Article

Public Attitude towards Biobanking: An Italian University Survey

Chiara Aleni ^{1,*}, Carmela Rinaldi ^{2,3,4}, Valentina Bettio ^{3,4}, Eleonora Mazzucco ^{3,4}, Annamaria Antona ⁴, Cristina Meini ¹, Emiliano Loria ⁴, Paolo Bonvicini ⁵, Silvia Vittoria Cracas ⁴, Silvia Caristia ⁴, Antonio Rimedio ⁶, Fabrizio Faggiano ¹, Daniela Ferrante ⁴ and Daniela Capello ^{3,4}

¹ Department of Sustainable Development and Ecological Transition, University of Piemonte Orientale, 13100 Vercelli, Italy

² “Maggiore della Carità” Hospital, 28100 Novara, Italy

³ UPO Biobank, University of Piemonte Orientale, 28100 Novara, Italy

⁴ Department of Translational Medicine, University of Piemonte Orientale, 28100 Novara, Italy

⁵ Biobanks and Complex Data Management, University of Cote d’Azur, 06108 Nice, France

⁶ Ethics Committee of the “Maggiore della Carità” Hospital, 28100 Novara, Italy

* Correspondence: chiara.aleni@uniupo.it

Abstract: Biobanks have established a critical role in biomedical research by collecting, preserving, organizing, and disseminating biospecimens and related health data, contributing to precision medicine development. Participation in biobanks is influenced by several factors, such as trust in institutions and scientists, knowledge about biobanking, and the consideration of benefit sharing. Understanding public attitudes, fears, and concerns toward biobanking is fundamental to designing targeted interventions to increase trust towards biobanks. The aim of our study was to investigate the level of knowledge and perception of biobanks in students and personnel of the University of Piemonte Orientale. An online questionnaire was designed and administered via e-mail. A total of 17,758 UPO personnel and students were invited to participate in the survey, and 1521 (9.3%) subjects completed the survey. The results showed that 65.0% of the participants were aware of the term “biobank” and knew what the activity of a biobank was, and 76.3% of subjects were willing to provide biospecimens to a research biobank, whereas 67.3% of the respondents were willing to contribute, in addition to biospecimens, their health and lifestyle data. Concerns were raised about the confidentiality of the information (25.6%) and the commercial use of the samples (25.0%). In conclusion, participants were aware of the role that biobanks play in research and were eager to participate for the sake of furthering scientific research. Still, several concerns need to be addressed regarding the confidentiality of the data along with the commercial use of the samples and associated data.

Keywords: biobanks; survey; population



Citation: Aleni, C.; Rinaldi, C.; Bettio, V.; Mazzucco, E.; Antona, A.; Meini, C.; Loria, E.; Bonvicini, P.; Cracas, S.V.; Caristia, S.; et al. Public Attitude towards Biobanking: An Italian University Survey. *Int. J. Environ. Res. Public Health* **2022**, *19*, 13041. <https://doi.org/10.3390/ijerph192013041>

Academic Editor: Paul B. Tchounwou

Received: 30 August 2022

Accepted: 9 October 2022

Published: 11 October 2022

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Personalized and precision medicine have acquired increasing attention over recent years. Advances in molecular medicine, genetics, and bioinformatics have made it possible to optimize prevention strategies, identify and tailor therapies, and determine predisposition to disease [1,2]. The success of personalized medicine requires a critical number of biological, genetic, health, and other personal data (i.e., lifestyle and socioeconomic data) organized according to scientific criteria and high-quality standards [3,4]. Research biobanks respond to this need by collecting, storing, and disseminating biospecimens along with the associated information to sustain high-impact biomedical research [5].

Human research biobanks can be distinguished according to the types of biological samples, such as DNA, plasma, and tissue samples, or the purpose, including disease-based (samples from patients with a specific disease), population-based, or project-driven

biobanks [6]. Population biobanks are a fundamental tool to carry out pioneering multidisciplinary research for the investigation of genetic, environmental, lifestyle, and socioeconomic factors associated with the state of health [7]. The functioning and the success of any biobank require the participation of many individuals willing to provide data and/or biological material [3]. Participation is influenced by many factors, such as the participant's knowledge about biobanking [8,9], trust in research institutions and scientists [9], consideration of benefit sharing [10], expected benefits, and personal beliefs (cultural and religious) [11]. At the same time, biobank activity entails several challenges, including the recruitment of the participants, data security, informed consent, data sharing, future research topics, the returning of results, and other legal, social, and ethical issues [12]. Understanding the public's attitude toward biobanking and the engagement of the population are key factors to developing more effective recruitment strategies [3,13]. Overall, studies published to date demonstrate that knowledge and perception of biobanks are still limited [3], and consequently understanding fears and concerns about the reliance on personal data and biological samples is fundamental for designing targeted interventions to spread information and awareness and increase trust toward biobanks.

The UPO Biobank is the institutional research biobank of the University of Eastern Piedmont (UPO). It was implemented in April 2020 and is dedicated to supporting aging research. The UPO Biobank was established as a multispecialty biobank with both a population- and disease-oriented commitment.

The aims of this study were to investigate the level of knowledge and perception of biobanks in students and personnel of the University of Eastern Piedmont, focusing on the perceived benefits and risks of biobanking and the willingness to participate and to test and optimize a survey aimed at investigating the perception and willingness to donate in the general population.

2. Materials and Methods

2.1. Study Design and Population

This was a cross-sectional, survey-based study. A questionnaire-based survey was conducted among students and personnel of the University of Eastern Piedmont to explore attitudes, concerns, and expectations towards biobanking and the willingness to provide samples/data for biobank activity. Around 17,000 students and university personnel were invited to participate in the survey via e-mail between August 2021 and December 2021. Participants were over 18, including students enrolled in any course and personnel (including administration and academics) of the University of Eastern Piedmont, and they had to sign the informed consent. The exclusion criterion was the absence of signed informed consent. The study protocol was approved by the UPO data protection officer and the local ethical committee.

2.2. Study Tool and Data Collection

An online Italian-language survey was implemented using the REDCap platform (REDCap, Vanderbilt University). The questionnaire consisted of three main sections: (a) sociodemographic data and information about trust in research and researchers; (b) knowledge about biobanks and their activities; and (c) a UPO Biobank activity description and willingness to participate in it. Questionnaire validation and improvement was carried out before the survey launch according to 10 researchers' feedback and evaluations.

Participants were invited to participate in the survey via e-mail. Those who decided to participate were invited to click a link in the e-mail to be redirected to the REDCap platform, where they found a short introductory text explaining how the data would be collected, stored, and anonymized. The participant's informed consent for data processing was mandatory to access the questionnaire section.

2.3. Measures and Questionnaire

Demographic Data. In this section, containing 22 items, participants were asked about their age, sex, nationality, role in the University (student, PhD student, research fellow, professor, or administrative/technical staff), their department of affiliation, and information about the family (occupation of father/mother). Moreover, information about the health status of the participants was collected (general health status and diagnosis of chronic conditions). Participants were also asked to rate their interest in scientific subjects, their knowledge of biomedical subjects, and their trust in scientists and research. As the survey was conducted during the peak of the second wave of the COVID-19 pandemic, participants were also asked about their interest and trust in science before and after the pandemic.

Biobanks and their activities. In this section, containing 10 items, participants were asked about their knowledge of biobank activity. Subjects were also interviewed about their willingness to provide biospecimens and personal data to biobanks and about concerns, including a lack of confidence in the usefulness of their sample or in the quality of the research, a fear that the sample would be used for commercial purposes, a fear of genetic research, a fear of needles, and the time spent participating. Furthermore, participants were questioned about their willingness to provide their personal data and what type of consent they were willing to provide.

Knowledge about the UPO Biobank. In the last section of the questionnaire, containing two items, participants were asked about their willingness to participate in UPO Biobank projects by providing biospecimens and personal data.

2.4. Statistical Analysis

Quantitative variables are presented as the median and interquartile range (IQR) because the data were not normally distributed. Categorical variables are summarized as counts and percentages. Differences in medians were evaluated using the Mann–Whitney test. Associations between categorical variables were tested using the Pearson χ^2 test. A two-sided p value < 0.05 was considered statistically significant. The analyses were performed using STATA software, version 17 (StataCorp. 2021 Statistical Software: Release 17; College Station, TX, USA, Stata Corporation).

2.5. Ethical Considerations

Subjects participating in the study were asked for informed consent. Confidentiality was ensured throughout all phases of the study. Personal data were anonymized and stored in REDCap software (Nashville, TN, USA), whose access was only granted to the members of the study in charge of data analysis. Data were aggregated before the analysis. All data were collected and treated according to the EU General Data Protection Regulation (GDPR) 2016/679.

3. Results

3.1. Demographics and Socioeconomic Characteristics

A total of 17,781 students and personnel, including 16,904 undergraduate and graduate students, 117 research fellows, 318 technical/administrative staff, and 382 researchers and professors were invited to participate in the survey. Overall, 1658 (9.3%) of the subjects entered the survey, whereas 1521 (8.5%) provided informed consent and completed it. Most of the participants were female (68.6%) and of Italian nationality (94.2%), in accordance with the sex and nationality distribution of the UPO population. Overall, most participants were represented by undergraduate and graduate students (77.9%), followed by researchers and professors (15.3%), and technical/administrative staff (6.8%). A significantly higher response rates were observed among professors (54.6%; $p < 0.0001$), researchers and research fellows (30.4%; $p < 0.0001$), technical/administrative staff (32.4%; $p < 0.0001$), and PhD students (12.2%; $p = 0.001$) compared to undergraduates (6.9%) [Table 1a].

Table 1. (a) Demographic characteristics of UPO students and personnel and those of participants. (b) Distribution of degree programs among UPO students.

(a)			
Respondents		UPO Students and Personnel	<i>p</i> -Value
Characteristic	<i>n</i> (%)	<i>n</i>	
Sex			
Male	477 (6.7)	7158	–
Female	1044 (9.8)	10,623	<0.0001
University role			
Students	1155 (6.9)	16,658	–
Ph.D. students	30 (12.2)	246	0.001
Research fellows	42 (23.7)	177	
Researchers	49 (40.2)	122	<0.0001
Professors	142 (54.6)	260	<0.0001
Administrative/technical staff	103 (32.4)	318	<0.0001
Total	1521 (8.5)	17,781	
(b)			
Respondents		UPO Students	<i>p</i> -Value
	<i>n</i> (%)	<i>n</i> (%)	
Business administration	81 (7.0)	2312 (13.9)	<0.0001
Biological science	157 (13.6)	2131 (12.8)	0.62
Biotechnology	220 (19.0)	1411 (8.5)	<0.0001
Medicine and Surgery	114 (9.9)	1007 (6.0)	0.0008
Medical Biotechnology	53 (4.6)	188 (1.1)	<0.0001
Nursing and Obstetrics	25 (2.2)	135 (0.8)	0.008
Philosophy	6 (0.5)	42 (0.2)	0.22
Total	1155	16,658	

Significantly higher than expected proportions of respondents were observed among medical biotechnology ($p < 0.0001$), nursing and obstetrics ($p = 0.008$), biotechnology ($p < 0.0001$), and medicine and surgery ($p = 0.0008$) students. On the contrary, business administration students demonstrated a significantly lower proportion of respondents than expected ($p < 0.0001$) (Table 1b).

3.2. Interest in Science and Scientific Research

Overall, participants showed a high interest in scientific disciplines and a deep trust in biomedical research and researchers (median = 9, IQR 8–10). A statistically significant difference in trust in biomedical research was found between the pre-COVID-19 period and during the COVID-19 pandemic (difference between pre- and post-COVID-19: $p < 0.0001$). Respondents rated the impact of scientific research on society as very important (median = 10, IQR = 9–10). Responses to the “risks and benefits of biomedical research” are detailed in Table 2. Most participants in the study (85.1%) stated that the benefits of biomedical research far outweigh the risks.

Table 2. Perception of benefits and risks associated with biomedical research.

Balance between Benefits and Risks	UPO Students <i>n</i> (%)	Personnel <i>n</i> (%)	Total <i>n</i> (%)
The benefits are greater than the risks.	908 (83.5)	290 (90.6)	1198 (85.1)
The benefits are slightly greater than the risks.	94 (8.6)	16 (5.0)	110 (7.8)
The benefits are equal to the risks.	72 (6.6)	11 (3.4)	83 (5.9)
The risks are slightly greater than the benefits.	5 (0.5)	1 (0.3)	6 (0.4)
The risks are greater than the benefits.	9 (0.8)	2 (0.6)	11 (0.8)
Total	1088 (100)	320 (100)	1408 (100)

3.3. Biobanks and Their Activities

Knowledge about biobanks and biobanking activities is summarized in Table 3. This section was briefly introduced by an explanation about biobanks and their role in research. More than half of the participants (65%) declared to have heard the term “biobank” before this survey and knew what a biobank does. The knowledge level about biobanks and research biobanks was significantly higher among university personnel compared with students ($p < 0.0001$). Most of the respondents (84%) knew about the presence of research biobanks in Italy.

Table 3. Knowledge about biobanks and biobanking activities, concerns, and informed consent.

Questions	Students <i>n</i> (%)	Personnel <i>n</i> (%)	Total <i>n</i> (%)	<i>p</i> -Value
Have you ever heard of the term research biobanks before?				
Never	349 (29.5)	54 (16.1)	403 (26.5)	<0.0001
Occasionally	574 (48.4)	172 (51.2)	746 (49.0)	
Often	152 (12.8)	92 (27.4)	244 (16.0)	
Missing	110 (9.3)	18 (5.3)	128 (8.4)	
Do you know which is the activity of research biobanks?				
No	351 (29.6)	56 (16.7)	407 (26.8)	<0.0001
Yes, vaguely	404 (34.1)	106 (31.5)	510 (33.5)	
Yes, quite	288 (24.3)	111 (33.0)	399 (26.2)	
Yes, very well	31 (2.6)	45 (13.4)	76 (5.0)	
Missing	111 (9.4)	18 (5.4)	129 (8.5)	
Do biobanks contribute to the advancement of biomedical knowledge?				
Medium score (Range 0–10) [IQR]	9 [8,9,10]	9 [8,9,10]	9 [8,9,10]	0.0006
Are you willing to donate biological samples to a biobank?				
Yes	896 (75.6)	265 (78.9)	1161 (76.3)	0.15
Yes, under some conditions	95 (8.0)	36 (10.7)	131 (8.6)	
No	68 (5.7)	13 (3.9)	81 (5.3)	
Missing	126 (10.6)	22 (6.5)	148 (9.7)	
Why would you donate a biological sample to a biobank? (more than one option)				
Contribute to scientific research	888 (74.9)	257 (76.5)	1145 (75.3)	–
Increase knowledge/benefit for future generations	687 (58.0)	178 (53.0)	865 (56.9)	
Sense of duty	282 (23.8)	71 (21.1)	353 (23.2)	
Benefit for a family member or friends	210 (17.7)	24 (7.1)	234 (15.4)	
Personal benefit	186 (15.7)	22 (6.5)	208 (13.7)	

Table 3. Cont.

Questions	Students <i>n</i> (%)	Personnel <i>n</i> (%)	Total <i>n</i> (%)	<i>p</i> -Value
What are your concerns about donating biological samples to a research biobank? (more than one option)				
Fear that the confidentiality of the information is not guaranteed	318 (26.8)	71 (21.1)	389 (25.6)	–
Fear that my biological sample is being used for commercial purposes	305 (25.7)	75 (22.3)	380 (25.0)	
Fear of needles/blood	147 (12.4)	19 (5.6)	166 (10.9)	
Lack of confidence in the usefulness of my sample for scientific research	66 (5.6)	10 (3.0)	76 (5.0)	
Fear of genetic research	49 (4.1)	20 (6.0)	69 (4.5)	
Little confidence in the quality of scientific research using my sample	53 (4.5)	14 (4.2)	67 (4.4)	
No time	47 (4.0)	17 (5.1)	64 (4.2)	
No concerns	377 (31.8)	134 (40.0)	511 (33.6)	
What form of consent would you give for the use of your biological sample if you choose to commit it to a research biobank?				
I would like to be asked for consent whenever a study will need to use my sample.	448 (37.8)	93 (27.7)	541 (35.6)	<0.0001
I would consent to the use of my sample for research, about which I would like to be informed, but without need to provide further consents.	494 (41.7)	163 (48.5)	657 (43.2)	
Once I have given the sample, I am not at all interested in knowing if and how it will be used.	47 (4.0)	36 (10.7)	83 (5.5)	
I do not know.	26 (2.2)	17 (5.1)	43 (2.8)	
Missing	170 (14.3)	27 (8.0)	197 (12.9)	
If you decide to donate a biological sample to a research biobank, would you also provide personal information?				
Yes, I would also provide information regarding health, lifestyle, and habits	787 (66.4)	237 (70.5)	1024 (67.3)	0.20
Yes, but I would only provide information about health and not information related to habits and lifestyle.	107 (9.0)	25 (7.4)	132 (8.7)	
No, I would only provide the biological sample.	64 (5.4)	19 (5.7)	83 (5.5)	
I do not know.	64 (5.4)	29 (8.6)	93 (6.1)	
Missing	163 (13.8)	26 (7.7)	189 (12.4)	

Participants demonstrated a high level of agreement with the sentence “research biobanks activities are important for the progress of biomedical research” (median = 9, IQR 8–10). In order to assess the level of trust in biobank activity, subjects were asked about their willingness to participate in biobanking. The majority of respondents (76.3%) were willing to provide biological samples to biobanks for research activity. The main reasons behind this choice were contributing to scientific research (75.3%), increasing knowledge in science for future generations (56.9%), and a sense of duty (23.2%). Only 5.3% of respondents, predominantly students, said they were unwilling to provide a biological sample to a biobank. None of them had any previous knowledge of biobanks.

To understand the factors that may affect participation in biobanks and biomedical research, respondents were questioned about concerns regarding the biobanking of biologic materials. Greater concerns were raised about the confidentiality of the information (25.6%), the commercial use of the samples (25.0%), and the fear of needles/blood (10.9%). Notably, almost all participants (96.0%) did not express any concern about genetic research.

Then, the willingness to provide personal information was explored. The majority (67.3%) of the participants were willing to provide health and lifestyle information, whereas only 5.5% of them were not inclined to give any information other than the biological samples.

Therefore, participants were asked about the level of information and control they would like to have over the use of their samples and data. Around half of the participants (43.2%) would like to know what research their sample would be used in, whereas 35.6% of respondents would prefer to be asked whenever their sample is used in a study.

4. Discussion

Our results clearly showed a high response rate among researchers and technical/administrative personnel and a very low response rate among students. The low rate among students could be explained by: the use of e-mail to administer the survey, the low interest in taking part in surveys, and the period of the study between August and December, a transitional period between the end of one academic year and the next. Higher rates were shown among scientific courses (biotechnology, biology, medicine, and surgery), as shown in other studies [5].

Overall, the majority (65%) of respondents among UPO students and personnel had a noteworthy level of knowledge of the meaning of the term “biobank” and the role that biobanks play in biomedical research. These results are in line with a study conducted in Jordan [5] in which 53% of university students had previous knowledge of the term biobank and of biobanking activities, whereas other studies conducted in Saudi Arabia and Russia reported levels of knowledge lower than 30% [14,15]. The high level of knowledge among the UPO population may be the consequence of local social media campaigns (including articles, interviews, and news) and, possibly, of their involvement in biobank research projects.

Most participants recognized the role of biobanks in research and, for this reason, were eager to provide biological samples to a biobank. In contrast with other studies [16], we did not find an association between sex and willingness to donate.

According to the results of a recent literature review [3], the advancement of biomedical research and benefits for society and future generations were the major reasons for the positive attitude toward participating in a biobank.

Concern about privacy, the risk of discrimination, the commercialization of samples, and associated data for profit seem to be major contributors to the hesitation in participating in a research biobank [3]. A quarter of participants raised a concern about the confidentiality of information (privacy) and the commercialization of biological samples. These were also the main concerns expressed in other studies [6,17–20], especially when genetic data are involved [13]. In our study the percentage of subjects willing to participate in biobanking decreased by about 10% when respondents were asked if they were willing to provide, in addition to biological samples, health and lifestyle data.

A biobank should inform healthcare professionals and the general population about the regulation under which they are treated and protected. Biobanks all over Europe are required to comply with the GDPR [21], which is widely implemented to address these concerns and grants protection to all EU citizens whenever their personal data are processed, with specific attention and derogations to health and genetic data processing (article 89 of the GDPR).

Of a different nature is the concern regarding the exploitation of samples for commercial purposes because participants consider such a purpose conflicting with their entirely disinterested choice (23.3%). In general, a higher trust in academic institutions than in private and/or profit organizations and biobanks has been reported [22–25], corroborating the key role of public academic institutions in contributing to scientific progress and health promotion but with the priority of protecting the human and legal rights of individuals. The UPO Biobank is a “non-profit service structure”, but this does not exclude that the obtained results can be exploited by private companies to improve health technologies and prepare new drugs because the population can benefit from the research only through these steps.

For all these reasons, a population biobank must match the ‘trust’ of citizens with the ‘trustworthiness’ of its governance: trustworthiness is an intrinsic ethical value and is also

instrumental in increasing research participation and improving the perception of research by the public.

Genetic research entails several concerns and potential risks for individuals; surprisingly, a very small percentage of respondents (4.0%) had concerns about genetic research and the only one was “the fear of discovering a genetic disease/being a carrier of a genetic disease” (2.3%). Genetic research, such as genomic and genetic profiling, could result in the discovery of information on rare or complex conditions beyond the research target, also called “incidental findings” (IFs) [26,27]. The chance of identifying significant or pathogenic IFs using whole genome sequencing (WES) has been reported to be around 1–6% in the adult population [28,29], and this percentage is likely to increase. Deciding how and when to report IFs has been thoroughly discussed among scientists. National and international laws and guidelines provide minimal clues to researchers [26]. For example, the American College of Medical Genetics and Genomics has identified a list of genes that should be reported as IFs [30,31], whereas other authors in the UK suggest that IFs should be returned only in the case of serious conditions [32,33]. Surveys involving patients and the general population report mixed results: in the USA, IFs have been well-received by patients, while opposite results were found in a similar European study [34].

Participants show a high interest in scientific research (75.3%) and in providing a benefit for future generations (56.9%). At the same time, many respondents would prefer to be informed for every use of the donated sample (35.5%, asking for a specific consent). This may indicate the need to maintain some control over the type of research their sample and information are used in.

Therefore, the custody of biological samples needs to be nurtured through a continuous ‘connection’ with the biobank, understood as a physical place for storing and processing samples and above all as a ‘research community’ that binds researchers and citizens in a bond of solidarity and mutual responsibility. The new means of information make it possible to ensure that each sample trustee has a personalized flow of information on possible collaborations and different projects within the previously selected research areas, allowing the participant to exercise their rights at any time or to oppose processing operations that do not comply with their reference values. These are the characteristic features of the “dynamic” consensus which, currently is the most adequate and flexible response to the needs of “connection” manifested by citizens.

The main limitation of this study is the limited generalization of results to the general population. As with many other similar studies, it involved a selected academic population, and the response rate, even if higher than those in many other studies, was quite low. The few studies involving the general population seem to show results that are not far from those coming from selected populations [17,20,35]. This suggests that the estimations made by our study could be used, even if with some degree of caution, to plan the involvement of the population in cohort studies based on biobanking.

In the last 10 years, around 60 biobank-related surveys have been conducted and published all over the world, with less than 20 being related to European biobanks. In Italy, few studies have been conducted in the last 10 years [16,36,37], with none being published in the last 3 years. Of the three studies conducted in Italy, just one [16], published in 2017, investigated the willingness to donate and the attitude towards biobanking among university students. In comparison, we found a higher willingness to donate (76.3% compared to 57.7%), and a higher percentage knew about the presence of biobanks in Italy (84% compared to 43%). For the first time in years, this survey gives insight into the knowledge and perception of Italian university personnel about biobanks and scientific research and highlights critical issues regarding the perception that citizens’ have about the protection of their personal data and the type of informed consent to be offered to participants.

5. Conclusions

In this article, we reported high levels of support and willingness to donate and contribute to a research biobank of students and personnel of the University of Piemonte Orientale. Participants were aware of the fundamental role that biobanks play in research and were keen to participate in them for the sake of advancing biomedical research. Nonetheless, a number of concerns need to be addressed regarding the confidentiality of the data and information along with the commercial use of the samples and associated data. A well-structured survey aimed at the general population and investigating particular aspects of biobanking, including the return of results, privacy, data sharing, informed consent, and the commercial use of samples and data, could better clarify how to address these concerns.

Author Contributions: Conceptualization, D.C., F.F., C.A., A.R., D.F., C.M., V.B., E.M., C.R. and S.C.; methodology, D.C., D.F. and C.R.; writing—original draft preparation, C.A., D.C., F.F. and C.R.; data curation, D.F., D.C., F.F. and C.A.; writing—review and editing, C.A., D.C., F.F., C.R., D.F., V.B., E.M., A.A., A.R., C.M., E.L., P.B., S.V.C. and S.C.; supervision, D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the University del Piemonte Orientale (FAR 2017 and FAR 2019) (D.C.), and by the Italian Ministry of Education, University, and Research (MIUR) program's Departments of Excellence 2018–2022, AGING Project—Department of Translational Medicine, University del Piemonte Orientale (D.C.).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: This research was partially supported by the donations of Fondazione Comunità Novarese, the Rotary Club Progetto Diabete and the Rotary Club Val Ticino.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Council of the European Union. Council conclusions on personalised medicine for patients. *Off. J. Eur. Union* **2015**, *15*, 2013–2016.
2. Lemke, A.A.; Wolf, W.A.; Hebert-Beirne, J.; Smith, M.E. Public and biobank participant attitudes toward genetic research participation and data sharing. *Public Health Genom.* **2010**, *13*, 368–377. [[CrossRef](#)] [[PubMed](#)]
3. Domaradzki, J.; Pawlikowski, J. Public attitudes toward biobanking of human biological material for research purposes: A literature review. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2209. [[CrossRef](#)] [[PubMed](#)]
4. Flores, M.; Glusman, G.; Brogaard, K.; Price, N.D.; Hood, L. P4 medicine: How systems medicine will transform the healthcare sector and society. *Pers. Med.* **2013**, *10*, 565–576. [[CrossRef](#)]
5. Khatib, F.; Jibrin, D.; Al-Majali, J.; Elhussien, M.; Almasaid, S.; Ahram, M. Views of university students in Jordan towards Biobanking. *BMC Med. Ethics* **2021**, *22*, 152. [[CrossRef](#)]
6. Abdelhafiz, A.S.; Sultan, E.A.; Ziady, H.H.; Sayed, D.M.; Khairy, W.A. Knowledge, perceptions and attitude of Egyptian physicians towards biobanking issues. *PLoS ONE* **2021**, *16*, e0248401. [[CrossRef](#)]
7. Coppola, L.; Cianflone, A.; Grimaldi, A.M.; Incoronato, M.; Bevilacqua, P.; Messina, F.; Baselice, S.; Soricelli, A.; Mirabelli, P.; Salvatore, M. Biobanking in health care: Evolution and future directions. *J. Transl. Med.* **2019**, *17*, 172. [[CrossRef](#)]
8. Gaskell, G.; Gottweis, H. Biobanks need publicity. *Nature* **2011**, *471*, 159–160. [[CrossRef](#)]
9. Godard, B.; Marshall, J.; Laberge, C. Community engagement in genetic research: Results of the first public consultation for the Quebec CARTaGENE project. *Community Genet.* **2007**, *10*, 147–158. [[CrossRef](#)]
10. Meulenkamp, T.M.; Gevers, S.K.; Bovenberg, J.A.; Koppelman, G.H.; van Vlieg, A.H.; Smets, E.M.A. Communication of biobanks' research results: What do (potential) participants want? *Am. J. Med. Genet. Part A* **2010**, *152*, 2482–2492. [[CrossRef](#)]
11. Lewis, C.; Clotworthy, M.; Hilton, S.; Magee, C.; Robertson, M.J.; Stubbins, L.J.; Corfield, J. Public views on the donation and use of human biological samples in biomedical research: A mixed methods study. *BMJ Open* **2013**, *3*, e003056. [[CrossRef](#)] [[PubMed](#)]
12. Haga, S.B.; Beskow, L.M. Ethical, Legal, and Social Implications of Biobanks for Genetics Research. *Adv. Genet.* **2008**, *60*, 505–544. [[PubMed](#)]
13. Shabani, M.; Bezuidenhout, L.; Borry, P. Attitudes of research participants and the general public towards genomic data sharing: A systematic literature review. *Expert Rev. Mol. Diagn.* **2014**, *14*, 1053–1065. [[CrossRef](#)] [[PubMed](#)]

14. Tsvetkova, L.A.; Eritsyan, K.Y.; Antonova, N.A. Russian students' awareness of and attitudes toward donating to biobanks. *Psychol. Russ. State Art* **2016**, *9*, 30–38. [\[CrossRef\]](#)
15. Merdad, L.; Aldakhil, L.; Gadi, R.; Assidi, M.; Saddick, S.Y.; Abuzenadah, A.; Vaught, J.; Buhmeida, A.; Al-Qahtani, M.H. Assessment of knowledge about biobanking among healthcare students and their willingness to donate biospecimens. *BMC Med. Ethics* **2017**, *2*, 18. [\[CrossRef\]](#)
16. Tozzo, P.; Fassina, A.; Caenazzo, L. Young people's awareness on biobanking and DNA profiling: Results of a questionnaire administered to Italian university students. *Life Sci. Soc. Policy* **2017**, *13*, 9. [\[CrossRef\]](#)
17. Mezinska, S.; Kaleja, J.; Mileiko, I.; Santare, D.; Rovite, V.; Tzivian, L. Public awareness of and attitudes towards research biobanks in Latvia. *BMC Med. Ethics* **2020**, *21*, 65. [\[CrossRef\]](#)
18. Kaufman, D.J.; Murphy-Bollinger, J.; Scott, J.; Hudson, K.L. Public Opinion about the Importance of Privacy in Biobank Research. *Am. J. Hum. Genet.* **2009**, *85*, 643–654. [\[CrossRef\]](#)
19. Kettis-Lindblad, Å.; Ring, L.; Viberth, E.; Hansson, M.G. Perceptions of potential donors in the Swedish public towards information and consent procedures in relation to use of human tissue samples in biobanks: A population-based study. *Scand. J. Public Health* **2007**, *35*, 148–156. [\[CrossRef\]](#)
20. Broekstra, R.; Maekelberghe, E.L.M.; Aris-Meijer, J.L.; Stolk, R.P.; Otten, S. Motives of contributing personal data for health research: (non-)participation in a Dutch biobank. *BMC Med. Ethics* **2020**, *21*, 1–11. [\[CrossRef\]](#)
21. European Parliament. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the Protection of Natural Persons with Regard to the Processing of Personal Data and on the Free Movement of Such Data, and Repealing Directive 95/46/EC (General Data Protection Regulation) (Text with EEA Relevance). 2016. Available online: <https://eur-lex.europa.eu/eli/reg/2016/679/oj> (accessed on 1 July 2022).
22. Master, Z.; Claudio, J.O.; Rachul, C.; Wang, J.C.Y.; Minden, M.D.; Caulfield, T. Cancer patient perceptions on the ethical and legal issues related to biobanking. *BMC Med. Genom.* **2013**, *6*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Dixon-Woods, M.; Kocman, D.; Brewster, L.; Willars, J.; Laurie, G.; Tarrant, C. A qualitative study of participants' views on re-consent in a longitudinal biobank. *BMC Med. Ethics* **2017**, *18*, 1–11. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Garrison, N.A.; Sathe, N.A.; Antommaria, A.H.M.; Holm, I.A.; Sanderson, S.C.; Smith, M.E.; McPheeters, M.L.; Clayton, E.W. A systematic literature review of individuals' perspectives on broad consent and data sharing in the United States. *Genet. Med.* **2016**, *18*, 663–671. [\[CrossRef\]](#)
25. Caulfield, T.; Rachul, C.; Nelson, E. Biobanking, Consent, and Control: A Survey of Albertans on Key Research Ethics Issues. In *Biopreservation and Biobanking*; Mary Ann Liebert Inc.: Larchmont, NY, USA, 2012; pp. 433–438.
26. Black, L.; Avard, D.; Zawati, M.; Knoppers, B.; Hébert, J.; Sauvageau, G. Funding considerations for the disclosure of genetic incidental findings in biobank research. *Clin. Genet.* **2013**, *84*, 397–406. [\[CrossRef\]](#)
27. Shkedi-Rafid, S.; Dheensa, S.; Crawford, G.; Fenwick, A.; Lucassen, A. Defining and managing incidental findings in genetic and genomic practice. *J. Med. Genet.* **2014**, *51*, 715–723. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Amendola, L.M.; Dorschner, M.O.; Robertson, P.D.; Salama, J.S.; Hart, R.; Shirts, B.H.; Murray, M.L.; Tokita, M.J.; Gallego, C.J.; Kim, D.S.; et al. Actionable exomic incidental findings in 6503 participants: Challenges of variant classification. *Genome Res.* **2013**, *25*, 305–315. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Yang, Y.; Muzny, D.M.; Xia, F.; Niu, Z.; Person, R.; Ding, Y.; Ward, P.; Braxton, A.; Wang, M.; Buhay, C.; et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA—J. Am. Med. Assoc.* **2014**, *312*, 1870–1879. [\[CrossRef\]](#)
30. Green, R.C.; Berg, J.S.; Grody, W.W.; Kalia, S.S.; Korf, B.R.; Martin, C.L.; McGuire, A.L.; Nussbaum, R.L.; O'Daniel, J.M.; Ormond, K.E.; et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet. Med.* **2013**, *15*, 565–574. [\[CrossRef\]](#)
31. Miller, D.T.; Lee, K.; Gordon, A.S.; Amendola, L.M.; Adelman, K.; Bale, S.J.; Chung, W.K.; Gollob, M.H.; Harrison, S.M.; Herman, G.E.; et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **2021**, *23*, 1391–1398. [\[CrossRef\]](#)
32. Darnell, A.J.; Austin, H.; Bluemke, D.A.; Cannon, R.O.; Fischbeck, K.; Gahl, W.; Goldman, D.; Grady, C.; Greene, M.H.; Holland, S.M.; et al. A Clinical Service to Support the Return of Secondary Genomic Findings in Human Research. *Am. J. Hum. Genet.* **2016**, *98*, 435–441. [\[CrossRef\]](#)
33. Ormondroyd, E.; Mackley, M.P.; Blair, E.; Craft, J.; Knight, J.C.; Taylor, J.C.; Taylor, J.; Watkins, H. "Not pathogenic until proven otherwise": Perspectives of UK clinical genomics professionals toward secondary findings in context of a Genomic Medicine Multidisciplinary Team and the 100,000 Genomes Project. *Genet. Med.* **2018**, *20*, 320–328. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Robinson, J.O.; Wynn, J.; Biesecker, B.; Biesecker, L.G.; Bernhardt, B.; Brothers, K.B.; Chung, W.K.; Christensen, K.D.; Green, R.C.; Kaufman, D.; et al. Psychological outcomes related to exome and genome sequencing result disclosure: A meta-analysis of seven Clinical Sequencing Exploratory Research (CSER) Consortium studies. *Genet. Med.* **2019**, *21*, 2781–2790. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Bossert, S.; Kahrass, H.; Streh, D. The public's awareness of and attitude toward research biobanks—A regional German survey. *Front. Genet.* **2018**, *9*, 190. [\[CrossRef\]](#)
36. Porteri, C.; Togni, E.; Pasqualetti, P. The policies of ethics committees in the management of biobanks used for research: An Italian survey. *Eur. J. Hum. Genet.* **2014**, *22*, 260–265. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Porteri, C.; Pasqualetti, P.; Togni, E.; Parker, M. Public's attitudes on participation in a biobank for research: An Italian survey. *BMC Med. Ethics* **2014**, *15*, 81. [\[CrossRef\]](#) [\[PubMed\]](#)

The Novara Cohort Study: Rationale, Objective and Preliminary Findings From an Italian Ageing Cohort Study

Chiara Aleni

University of Eastern Piedmont Amadeo Avogadro

Silvia Cracas

`silvia.cracas@uniupo.it`

University of Eastern Piedmont Amadeo Avogadro

Giulia Garro

University of Eastern Piedmont Amadeo Avogadro

Annamaria Antona

University of Eastern Piedmont Amadeo Avogadro

Jacopo Venetucci

University of Eastern Piedmont Amadeo Avogadro

Valentina Bettio

University of Eastern Piedmont Amadeo Avogadro

Marco Varalda

University of Eastern Piedmont Amadeo Avogadro

Lorenza Scotti

University of Eastern Piedmont Amadeo Avogadro

Daniela Ferrante

University of Eastern Piedmont Amadeo Avogadro

Roberta Rolla

University of Eastern Piedmont Amadeo Avogadro

Fabrizio Faggiano

University of Eastern Piedmont Amadeo Avogadro

Daniela Capello

University of Eastern Piedmont Amadeo Avogadro

Article

Keywords: geroscience, biomarkers, epidemiology, longevity, health trajectories, longitudinal analysis, predictive modeling, age-related diseases, aging dynamics

Posted Date: October 21st, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4939105/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: Competing interest reported. Declaration of Conflicting Interests We disclose the following potential conflicts of interest: Professor Daniela Capello, the study supervisor, is the scientific director of UPO Biobank (Center for Autoimmune and Allergic Diseases, Novara, Italy), which supports this research and collects the biological samples. Additionally, several collaborators involved in this study hold positions within the Biobank. Nonetheless, we affirm that comprehensive measures were implemented to maintain objectivity and uphold scientific integrity.

Abstract

Background

The Novara Cohort Study (NCS) is the first multidisciplinary cohort study on aging in Northern Italy. It is designed to explore aging trajectories and health outcomes in the general population. This study involves the collection of biological samples and extensive data, including socioeconomic, medical history, lifestyle habits, quality of life and physical function.

Objective

This paper outlines the rationale, objectives, and preliminary findings of the NCS pilot phase. It discusses the baseline characteristics, initial biological characterization, and identifies key areas for improvement to ensure the successful implementation of the full-scale study.

Methods

The NCS pilot phase enrolled participants aged 35 and older residing in Novara, Italy. The study involved the collection of biological samples, medical examinations, questionnaires and functional tests. Data were collected included demographic information, physical activity, sleep quality, diet, quality of life, mental health, medical history, and medication use. Key blood parameters were analyzed alongside clinical data.

Results

The pilot phase enrolled 123 participants, 68 (55.3%) females and 55 (44.7%) males with a median age of 65 years old. The NCS pilot participants had higher education levels, lower smoking rates, and higher physical activity levels than the general population. Blood biomarker profiling showed significant variability across participants, providing a solid foundation for effectively analyzing aging trajectories.

Conclusions

The NCS pilot provided crucial insights into participant characteristics and identified areas for study protocol enhancement throughout all phases. These findings will guide refinements to optimize future study processes and outcomes, ultimately aimed at investigating the biological, social, and environmental determinants of aging in the Northern Italy area population.

INTRODUCTION

While people worldwide are experiencing increased longevity, this positive trend is accompanied by a rise in the portion of life spent dealing with chronic diseases and disability^{1,2}. The expanding unhealthy ageing population presents a growing challenge, potentially leading to escalating social and medical costs^{3,4}. Consequently, there is a need to develop interventions capable of preventing or delaying the onset of frailty and disease, extending the duration of a healthy lifespan. Accelerating the development of such interventions can be achieved through predictive information on subjects' biological age and by understanding determinants associated with healthy longevity⁵.

The ageing process is influenced by a multifaceted interplay among various factors including the environment (exposome), lifestyle choices, socio-economic conditions, and individual susceptibility⁶. Given the intricate nature of these interactions, population-based prospective studies serve as a strategic resource for comprehending ageing and its various trajectories⁷.

The Novara Cohort Study (NCS) is the first population-based, multidisciplinary longitudinal study on aging in Northern Italy, established to identify the biological, social, and economic determinants of aging trajectories. The findings will contribute to inform strategies for stakeholders and policymakers, guiding the design of interventions aimed at promoting healthier ageing in the Novara area and beyond. To achieve this goal, the NCS will gather biological samples alongside a diverse range of data, including medical history, lifestyle, habits, quality of life, and physical function assessments from a minimum of 10,000 participants aged 35 or older residing in the Novara province, located in Northern Italy. Biological samples will undergo a comprehensive array of analyses encompassing serological, genetic, epigenetic, proteomic, and metabolomics profiling. Utilizing advanced computational techniques, these datasets will be integrated with questionnaire information and health outcomes to unveil the complex relationships among the investigated determinants, thus contributing to a deeper understanding of ageing processes and the development of risk indicators based on the identified profiles.

This paper aims to describe the baseline characteristics of the population enrolled in the pilot study, evaluate its representativeness and lay the groundwork for identifying biomarkers capable of revealing subclinical deficits to predict different aging trajectories.

METHODS

Target population

The NCS is a longitudinal population study aiming to include a minimum of 10,000 participants aged 18 or older, representative of the Novara Province residents. Situated in northwest Italy within the industrialized region known as the 'Pianura Padana,' Novara Province spans 88 municipalities and is positioned between two of Italy's largest and most polluted metropolises, Milan and Turin. As of January 2023, the Novara Province had 362,502 residents, with a demographic breakdown of 48.8% male and 51.2% female, an average age of 46.9 years, and an aging index of 201. Approximately 25% of the population is over 65 years old, including 8% who are over 80 years old.

Participation process

The participation to the study is voluntary and a mass media campaign was established to inviting the population of the Novara Province. NCS investigators met with stakeholders, including general practitioners, healthcare professionals, municipality council representatives, and citizens' associations, to present the study and its objectives. Public meetings were also organized to engage with the general population, allowing citizens to express their willingness to participate directly at these events or by applying through the website.

The pilot study commenced in November 2022 and continued until May 2023, including subjects aged over 35 years. From November 2023, the NCS entered in the full implementation phase.

Ethical considerations and privacy

The study protocol was approved by the local Ethical Committee (Comitato Etico Interaziendale AOU Maggiore della Carità di Novara, Protocol Number CE137/2022). Participants were required to provide informed consent, ensuring their voluntary participation and understanding of the study's purpose and procedures. All data collection and management procedures adhere to the guidelines outlined in the EU General Data Protection Regulation (GDPR) 2016/679.

Participant Assessment Procedures

Participants in the pilot study underwent a comprehensive assessment, which encompassed the collection of biological samples, questionnaires, medical examinations, and functional and cognitive tests, with the entire process taking up to four hours. The assessment team consisted of a medical doctor, a nurse practitioner, and three researchers of the NCS staff. The testing center was in Novara, within the rooms of the University of Piemonte Orientale (UPO) research biobank (UPO Biobank). The use of the UPO Biobank outpatient clinic and laboratories ensured adherence to appropriate ethical and quality standards for all protocol procedures and the collection of high-quality biological samples and data.

Biological samples

Blood, saliva, and urine samples were collected, processed, and stored in UPO Biobank. Participants autonomously collected their saliva and urine samples, while a trained nurse collected the blood samples following standardized procedures. Blood collection occurred after the participants had fasted overnight. Upon completion of the biospecimen collection, breakfast was offered to all participants. Approximately 50 ml of blood was drawn from each participant, using a variety of tubes to accommodate different analyses and storage requirements (Table 1): i) Ethylenediaminetetraacetic acid (EDTA) tubes, used for fresh hematological analyses, as well as for biobanking dedicated to nucleic acid extractions, peripheral blood mononuclear cells (PBMC) cryopreservation, and plasma proteomic and metabolomic analyses; ii) Lithium Heparin (LH) tubes, for plasma dedicated to biochemical analyses and

biobanking; iii) Na-Citrate-containing tubes, for coagulation tests; iv) Gel serum separator tubes, for easy separation of serum from the clot, dedicated to serological and immunological analyses.

A portion of the fresh blood samples was immediately subjected to a series of hematological and biochemical assessments. The panel includes biomarkers associated with electrolyte balance, inflammation, cardiovascular disease risk, as well as indicators of renal damage, bone marrow, thyroid, and liver function (Table 1). Participants received the results of these tests, along with guidance on how to discuss them with their General Practitioner.

Table 1
Biospecimens: collection tubes, volumes, blood analyses on fresh samples, and biobanking

Collection tubes	Volume (ml)	Material	Blood biomarkers on fresh samples	Biobanking		
				N° of aliquots	Aliquots volume (µl)	Storage temp. (°C)
Serum gel (BD Vacutainer)	15	Serum	CYSC, protein electrophoresis, TP	4	500	-180
K2-EDTA (BD Vacutainer)	16	EDTA plasma	WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV, PDW, MPV, P-LCR, PLT, NEU, LYMPHO, MONO, EOS, BASO, EB, IG, HbA1c	6	500	-180
		EDTA buffy coat		1	300	-80
		Whole blood in RNA later		2	1600	-20
		PBMC		2	500 (2.0 mln)	-180
Sodium-Citrate (BD Vacutainer)	3	Citrate plasma	FBG, DD	4	500	-180
		Citrate buffy coat		2	300	-80
Gel Lithium-Heparin (BD Vacutainer)	13.5	Heparin plasma	ALT, AST, BIL, BUN, Ca, TC, CR, FER, FA, ALP, P, GGT, HDL, IL-6, LDL, CRP, K, Na, FT4, TRF, TG, TSH, UA, B12	6	500	-180
Vacutest (Securlab)	80–100	Urine		10	5000; 500	-80
Salivette (Sarstedt)	0.5	Saliva				-80

ALT, Alanine Aminotransferase; **ALP**, Alkaline Phosphatase; **AST**, Aspartate Aminotransferase; **BASO**, Basophils; **BIL**, Bilirubin; **BUN**, Blood Urea Nitrogen; **CA**, Calcium; **CR**, Creatinine; **CRP**, C-Reactive Protein; **CYSC**, Cystatin C; **DD**, D-dimer; **EB**, Erythroblasts; **EOS**, Eosinophils; **FA**, Folic Acid; **FBG**, Fibrinogen; **FER**, Ferritin; **FT4**, Free-thyroxine (FT4); **GGT**, Gamma-Glutamyl transferase; **HbA1c**, Glycated Hemoglobin; **HDL**, High-Density Lipoprotein; **HCT**, Hematocrit; **HGB**, Hemoglobin; **IG**, Immature Granulocytes; **IL-6**, Interleukin-6; **K**, Potassium; **LDL**, Low-Density Lipoprotein; **LYMPHO**, Lymphocytes; **MCV**, Mean Corpuscular Volume; **MCH**, Mean Corpuscular Hemoglobin; **MCHC**, Mean Corpuscular Hemoglobin Concentration; **MONO**, Monocytes; **MPV**, Mean Platelet Volume; **Na**, Sodium; **NEU**, Neutrophils; **P**, Phosphorus; **PDW**, Platelet Distribution Width; **PLT**, Platelets; **P-LCR**, Platelet Larger Cell Ratio; **RBC**, Red Blood Cells; **RDW-CV**, Red Cell Distribution Width-Coefficient of Variation; **RDW-SD**, Red Cell Distribution Width-Standard Deviation; **TSH**, Thyroid Stimulating Hormone; **TP**, Total Proteins; **TRF**, Transferrin; **UA**, Uric Acid; **WBC**, White Blood Cells.

Questionnaires

The dimensions investigated in the questionnaires included: i) demographic information: age, sex, place of birth, ethnicity, education level, marital status, occupational history and occupational exposure; ii) physical activity, assessed using the International Physical Activity Questionnaire (IPAQ)⁸; iii) sleeping habits, evaluated with the Pittsburgh Sleep Quality Index (PSQI)⁹ and the Epworth Sleepiness Scale (ESS)¹⁰, investigating snoring frequencies, nocturnal apnea, daytime sleepiness, frequency of unrested sleep and the presence of obstructive sleep apnea syndrome (OSAS); iv) smoking habits, investigated using a novel questionnaire developed and validated by the NCS staff; v) information about diet, including alcohol consumption, examined using a modified version of the food-frequency EPIC questionnaire¹¹; vi) quality of life, including life satisfaction, physical and psychological health and social behavior; vii) mental health, encompassing depression, anxiety and attachment style, and measured using the Beck Depression Inventory-II (BDI)¹², Beck Anxiety Inventory (BAI)¹³ and Attachment Style Questionnaire (ASQ)¹⁴ respectively.

Medical examination

The medical examination included an anamnestic interview and a physical assessment, conducted by healthcare professionals. The anamnestic interview explored personal and family medical histories, with diseases classified according to the International Classification of Diseases, 10th Revision (ICD-10). Information on current medication use was collected and coded using the Anatomical Therapeutic Chemical (ATC) classification system. Physical and cognitive assessments were performed using the Clinical Frailty Scale (CFS)¹⁵ and the Montreal Cognitive Assessment (MOCA)¹⁶. Anthropometric measurements such as height, weight, body mass index (BMI), waist-to-hip ratio (WHR), as well as vital signs including blood pressure, heart rate, respiratory rate, and blood oxygen saturation, were also recorded.

Functional tests

Several physical tests were used to assess the functional status, physical capabilities, and mobility of participants. The tests included: i) handgrip test, which measures upper body strength and muscle function; ii) Timed Up and Go Test (TUG)¹⁷, to assess mobility and balance based on the time to stand, walk, turn, and sit; iii) 6-Minute Walking Test¹⁸, to evaluate aerobic capacity and endurance by measuring walking distance; iv) Short Physical Performance Battery (SPPB)¹⁹, that provides a composite score of balance, gait speed, and lower extremity strength.

Follow-up

The NCS employs both passive and active follow-up methods. Passive follow-up consists of the periodical linkage with the clinical records and death register of the Novara Local Health Authority. Active follow-up is based on participants' age: those < 65 years undergo active follow-ups every 5 years, while those ≥ 65 years undergo active follow-ups every 3 years.

Data collection and analysis

The study utilized REDCap version 14.0.33 for secure pseudonymized data storage.

Descriptive analyses presented categorical variables as counts and percentages, while continuous variables were reported using mean and standard deviations (SD) for normally distributed variables or median and interquartile ranges (IQR) for skewed variables. Pearson's chi-square test was used to verify associations between categorical variables.

NCS pilot demographic and lifestyle characteristics were compared to those obtained from the PASSI (Progressi delle Aziende Sanitarie per la Salute in Italia) surveillance system. PASSI employs a random sampling technique for collecting information about lifestyle and risk factors for non-communicable diseases (NCDs) among the general adult population. For this study, data from residents in the Local Health Authority (LHA) of Novara between 60–69 years old were retrieved.

The Student's t-test and Mann-Whitney U test were employed to analyze the differences in quantitative variables between NCS subgroups, depending on the normality of the distributions as determined by the Shapiro-Wilk test. The Fisher-Pearson skewness test assessed the symmetry of the variables within their normal ranges. The Pearson coefficient was calculated to investigate relationships between variables after Z-score normalization. Partial least squares-discriminant analysis (PLS-DA) identified blood biomarkers distinguishing subjects with cardiovascular disease, diabetes, and different age groups. Variable Importance in Projection (VIP) analysis, with a threshold of $VIP > 1$, was conducted to identify the most influential variables driving the observed separation in the model. Statistical analyses were performed using STATA version 17, R version 4.4.0, and MetaboAnalyst 6.0. All tests were two-tailed, with a type I error set to 0.05.

RESULTS

Demographic and lifestyle characteristics of NCS pilot study participants

An overview of the NCS pilot participants' demographic characteristics is provided in Table 2. Overall, the NCS pilot study included 123 participants, with 68 (55.3%) women and 55 (44.7%) men; 45.5% of the participants were under 65, while 54.5% were 65 or older. Most participants had an upper secondary school diploma, with 45.4% of men and 55.9% of women receiving this qualification. Most participants were married (men 80%, women 58.8%), living with someone (men 85.5%, women 79.4%), and retired (men 67.3%, women 66.2%).

Lifestyle characteristics and risk factors were collected and analyzed (Table 3). Most participants identified themselves as either non-smokers or former smokers (76.4% of men and 95.6% of women). Almost all identified as omnivorous (92.7% of men and 89.7% of women). Adherence to a vegetarian diet was reported by 5.9% of women. Regarding alcohol consumption, the majority of NCS pilot participants (70.9% of men and 82.3% of women) reported consuming less than one alcoholic unit daily. Data on physical activity using the IPAQ questionnaire indicated that most of the NCS pilot population was at least moderately active (71.0% of men and 64.7% of women). The Pittsburgh Sleep Quality Index (PSQI) revealed that most NCS pilot participants experienced poor sleep quality (56.4% of men and 60.3% of women).

Table 2
NCS pilot participant demographic characteristics

NCS participants			
	Men n = 55	Women n = 68	Total n = 123
Age (years)-mean (SD)	65.5 (10.0)	64.7 (8.1)	65.1 (9.0)
Age ≤ 65			
Mean (SD)	56.5 (7.2)	58.8 (6.1)	57.9 (6.6)
Median (min-max)	57 (44–65)	61.5 (36–65)	61 (36–65)
Age > 65			
Mean (SD)	72.0 (5.7)	71.4 (4.0)	71.7 (4.9)
Median (min-max)	71 (66–93)	71 (66–80)	71 (66–93)
Education	n (%)	n (%)	n (%)
Primary or lower middle school	9 (16.4)	9 (13.2)	18 (14.7)
High school	25 (45.4)	38 (55.9)	63 (51.2)
University degree	21 (38.2)	21 (30.9)	42 (34.1)
Marital status			
Single, divorced or widower	10 (18.2)	27 (39.7)	37 (30.1)
Married	44 (80.0)	40 (58.8)	84 (68.3)
Missing	1 (1.8)	1 (1.5)	2 (1.6)
Cohabitation status			
Living alone	8 (14.5)	14 (20.6)	22 (17.9)
Cohabiting	47 (85.5)	54 (79.4)	101 (82.1)
Employment status			
Employed	18 (32.7)	15 (22.0)	33 (26.8)
Unemployed	0 (0.0)	8 (11.8)	8 (6.5)
Retired	37 (67.3)	45 (66.2)	82 (66.7)

n, number; **SD**, standard deviation.

Table 3
NCS pilot participant lifestyle and risk factors

NCS participants			
	Men	Women	Total
	n = 55	n = 68	n = 123
	n (%)	n (%)	n (%)
Smoking habits			
Never smoker	21 (38.2)	34 (50.0)	55 (44.7)
Past smoker	21 (38.2)	31 (45.6)	52 (42.3)
Current smoker	9 (16.3)	2 (2.9)	11 (8.9)
Missing	4 (7.3)	1 (1.5)	5 (4.1)
Alcohol consumption			
Never to seldom	11 (20.0)	23 (33.8)	34 (27.6)
< 1 alcohol unit/day	28 (50.9)	33 (48.5)	61 (49.6)
≥ 1 alcohol unit/day	9 (16.4)	7 (10.3)	16 (13.0)
Missing	7 (12.7)	5 (7.4)	12 (9.8)
Physical activity			
Low	11 (20.0)	19 (27.9)	30 (24.4)
Moderate	23 (41.9)	27 (39.7)	50 (40.7)
High	16 (29.1)	17 (25.0)	33 (26.8)
Missing	5 (9.1)	5 (7.4)	10 (8.1)
Participation in screening programs			
No	25 (45.5)	2 (2.9)	27 (21.9)
Yes	30 (54.5)	66 (97.1)	96 (78.1)
BMI			
< 25	28 (50.9)	33 (48.6)	61 (49.6)
25-29.99	20 (36.4)	24 (35.3)	44 (35.8)
≥ 30	7 (12.7)	10 (14.6)	17 (13.8)
Missing	-	1 (1.5)	1 (0.8)

NCS participants			
	Men	Women	Total
	n = 55	n = 68	n = 123
	n (%)	n (%)	n (%)
Diet			
Omnivorous diet	51 (92.7)	61 (89.7)	111 (90.2)
Vegetarian diet	0	4 (5.9)	4 (3.3)
Other	1 (1.8)	1 (1.5)	2 (1.6)
Missing	3 (5.5)	2 (2.9)	5 (4.1)
Sleep Quality			
Poor	31 (56.4)	41 (60.3)	72 (58.6)
Good	18 (32.7)	24 (35.3)	42 (34.1)
Missing	6 (10.9)	3 (4.4)	9 (7.3)

n, number; BMI, Body Mass Index

Health status of the NCS pilot study participants

Common cardiovascular disease (CVD) risk factors were evaluated (Table 3; **Supplementary Table 1**). Almost one in two participants was overweight (BMI ≥ 25), in particular 49.1% of men and 49.9% of women. The average systolic blood pressure (BP) recorded during the visits was 122.9 (13.6) mmHg for men and 123.8 (15.8) mmHg for women, both slightly above the recommended level (< 120 mmHg). In contrast, diastolic BP remained within the normal range (< 80 mmHg), averaging 76.4 mmHg for men and 77.3 mmHg for women.

Among the participants, 29.1% of men and 42.6% of women, reported a diagnosis of hypertension. Despite all of them claiming full adherence to antihypertensive therapy, elevated blood pressure readings were found in 47.0% of men and 55.2% of women during the visit. On the other hand, of those who did not report having hypertension, 42.2% of men and 41.0% of women still exhibited blood pressure readings above the normal range (*Data not shown*).

Forty percent of men and 27.9% of women reported having one chronic disease, while 36.4% of men and 42.7% of women declared having two or more conditions. The most frequent conditions were endocrine and metabolic diseases (34.5% of men and 57.4% of women), followed by cardiovascular disease (30.9% of men and 25% of women), and neoplasia (16.4% of men and 22.1% of women). Osteoporosis was reported by 1.8% of men and 29.4% of women (*Data not shown*).

Women reported a higher (83.9%), albeit not statistically significant, use of over-the-counter (OTC) and prescription medications compared to men (70.9%). The most used drug classes were antihypertensives (27.3% of men and 35.3% of women), endocrine therapy (14.5% of men and 22.1% of women), lipid-lowering drugs (10.9% of men and 11.8% of women), antithrombotic drugs (16.4% of men and .3% of women), and beta-blockers (10.9% of men and 10.3% of women) (*data not shown*).

Representativeness of the NCS pilot population compared to the general population

To evaluate the representativeness of the NCS pilot sample, we compared the demographic and lifestyle characteristics of the NCS pilot study participants with those of residents aged 60–69 in the ASL Novara, as derived from the PASSI surveillance system²⁰ (**Supplementary Table 2**). Compared with PASSI data, the NCS pilot participants had a significantly higher level of education ($p < 0.001$). Specifically, only 14.7% of NCS participants had primary or lower middle school education compared to 55.5% in PASSI; 51.2% had high school education compared to 34.9% in PASSI; and 34.1% had a university degree compared to 9.6% in PASSI. In terms of smoking habits, the NCS pilot participants exhibited a significantly lower prevalence of current smokers (8.9% vs. 17.0%, $p = 0.04$).

Alcohol consumption data showed that NCS pilot participants drank more alcohol, with 13.0% consuming ≥ 1 alcohol unit/day compared to 5.2% in PASSI ($p = 0.009$). Conversely, those consuming < 1 alcohol unit/day were more prevalent in PASSI than in the NCS pilot (77.2% vs 94.8%). Physical activity levels revealed a significantly higher proportion of individuals with low physical activity in the PASSI group (45.1%, $p < 0.001$) compared to the NCS pilot group (24.4%). Moderate activity levels showed no significant difference between the groups (40.7% in NCS vs. 32.4% in PASSI), nor did high activity levels (26.8% in NCS vs. 22.5% in PASSI). There were no significant differences between the NCS pilot and PASSI data in terms of marital status, cohabitation, employment status, participation in cancer screening programs, or BMI category distribution.

Identifying biomarkers to predict aging trajectories: insights from the NCS pilot

To assess the general health status of NCS participants, we identified a panel of 67 blood parameters through a literature review focusing on ageing. Descriptive statistics of these biomarkers are in **Table S3**.

Overall, 13 parameters were deranged in over 15% of participants (**Table S4**). Hypercholesterolemia (TC) was the most prevalent condition, affecting 72/123 (58.5%) subjects (mean: 229 mg/dL, SD: 21.9); 6 of them (8.3%) had a previous report, while 66 (91.7%) were newly identified. Mildly reduced renal function (< 90 mL/min/1.73 m²), indicated by a decreased estimated Glomerular Filtration Rate (eGFR) based on serum creatinine (CR) levels, was reported in 63 participants (51.2%) (**Table S5**) (median: 79.0, IQR: 67.0–86.5). Among these, 32 participants (50.8%) also exhibited abnormal cystatin C (CYSC) and/or blood urea nitrogen (BUN) values (*data not shown*). Conjugated (direct) hyperbilirubinemia (DBIL) was observed in 51/123 (41.5%) individuals (median: 0.3 mg/dL; IQR: 0.3–0.4 mg/dL). Twelve (23.5%)

participants also presented alteration in gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) hepatic markers (data not shown). Alterations in inflammatory biomarkers, including low transferrin (TRF), high interleukin-6 (IL-6), and/or high fibrinogen (FBG), were observed in 52.8%, 26.0%, and 19.5% of participants, respectively. Among these, 22.8% exhibited abnormalities in at least two out of the three parameters. Notably, among the biomarkers most affected, the distribution of values in individuals within the normal range frequently clustered close to their upper (e.g., TC, FBG, DBIL, and CYSC) or lower limits (e.g., eGFR) of the normal range (**Table S6**). This skewed distribution suggests early stages of biomarker dysregulation warranting closer monitoring and further investigation.

Next, to explore interactions within biological systems and uncover underlying relationships among blood parameters, we conducted pairwise evaluations to identify potential direct or inverse associations between variables. The Pearson correlation heatmap (Fig. 1; **Table S7**) displayed positive correlations among markers of liver function (GGT, ALP, and alanine aminotransferase/ALT), kidney function (CysC and CR) and hematological parameters, including hemoglobin (HGB), hematocrit (HCT), and red blood cells (RBC). We also noted positive correlations among inflammatory markers, including C-reactive protein (CRP), FBG, D-dimer (DD), alpha-1 (A1), alpha-2 (A2), and beta-1 (B1) proteins. These findings highlight the complex interplay and clinical relevance of these relationships, with implications for predicting aging quality and age-related disease risk, underscoring the need for further investigation in NCS follow-up.

Given that blood-based biomarker changes may be linked to various diseases in our population and/or aging, we examined the distribution of blood parameters concerning prevalent chronic diseases categorized by the ICD-10 classification system. Specifically, lower platelet levels (PLT) and ALP, alongside increased monocyte (MONO) and albumin (ALB) levels, were significantly associated with CVD, excluding hypertension (**Table S8**). Further analysis using PLS-DA and VIP scores identified MONO, PLT, vitamin B12 (B12), and ALP as key analytes contributing to the discrimination of this group of individuals (VIP score ≥ 2) (Fig. 2A-B).

Participants with diabetes were characterized, as expected, by elevated levels of glycated hemoglobin (HbA1c). Additionally, they showed elevated white blood cell counts (WBC), including neutrophils (NEU) and lymphocytes (LYMPH), as well as triglycerides (TG) and B1, alongside reduced levels of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) (**Table S9**). Variables with VIP greater than 1.5 included HbA1c, TG, WBC, and HDL (Fig. 2C-D).

To fulfill the ultimate objective of the NCS project, we explored the distribution of blood analytes related to age. Toward this aim, we grouped participants into those over and under 65 years old. Statistically significant differences were identified in several variables, including a decrease in eGFR and increases in AST, DD, CysC, and HbA1c among participants over 65 years old (**Table S10**). Variables with VIP scores greater than 1.4 characterizing the over-65 population included decreased eGFR and increased HbA1c, ferritin (FER), BUN, and CysC (Fig. 2E-F).

The graph visually represents the relationships between each pair of variables. The strength of each correlation is indicated by the Pearson correlation coefficient. Blue values denote negative correlations, while red values signify positive correlations. Lighter colors represent weaker correlations.

The scatter plot visually represents the separation of subjects based on the variables that distinguish them: the CVD group (in red) from the NO-CVD group (in green) **(A)**, the group of diabetic (in red) from the group of non-diabetic subjects (in green) **(C)**, and the group of over 65 years old (in red) from the group of under 65 years old (in green) individuals **(E)**. Each data point corresponds to a participant, and the placement of these points shows their projection onto the discriminant components. Ordered list included, showing analytes with higher discriminative power based on variable importance in projection (VIP) scores for distinguishing individuals with and without CVD **(B)**, with and without diabetes **(D)**, and under and over 65 years old **(F)**. The classification achieves statistical significance for VIP scores > 1.

DISCUSSION

The Novara Cohort Study (NCS) aims to comprehensively investigate both longitudinal and cross-sectional aspects of ageing within the Novara population. Dimensions considered include biological, physical, cognitive, environmental, social, and psychological aspects.

This pilot represents the first wave of the NCS study, which is instrumental in identifying areas for improvement across all phases of the protocol. These include refining public engagement and participation procedures, determining the most appropriate questionnaires and functional tests to administer, and optimizing biobanking and sample analysis procedures. Moreover, this initial phase allowed researchers to evaluate the representativeness of the population involved, as well as the predictive potential of blood biomarkers profiling.

NCS pilot participants, compared to the general population, had significantly higher education levels and physical activity. This is known as self-selection or volunteer bias, which is common in research involving volunteers, where individuals with higher education are more likely to participate²¹. This bias can limit the study's external validity, as volunteers may diverge significantly from the general population in ways that affect study outcomes²².

The NCS pilot results highlight the active involvement of health-focused citizens committed to supporting scientific research. Since external validity is a key objective of the NCS study, in the next phase a strategy to invite a random sample of the population will be implemented. On the other hand, participatory research included public events to promote community engagement. These activities raised trust, which is crucial for the success and sustainability of cohort studies²³.

The NCS pilot also provided an initial overview of the potential value of blood-based biomarkers by analyzing a panel of 67 biochemical parameters related to organ function, electrolyte balance, and inflammation. These parameters were chosen for their value in assessing frailty, their common use in clinical practice, and the availability of routine analytical procedures in hospital facilities^{24–26}.

Overall, our findings indicate that a significant proportion of the study participants exhibited alterations in biomarkers related to liver function, cardiometabolic health, and inflammatory conditions, with TC, DBIL, TRF, and eGFR being the most affected parameters. Hypercholesterolemia emerged as the most prevalent marker, indicating a considerable burden of subclinical or clinical cardiovascular conditions. These findings align with the Moli-SANI cohort, which highlighted a burden of cardiovascular conditions in the general population²⁷. Increased DBIL, shown in one in two participants, along with altered GGT, ALT, and ALP, suggests potential liver impairment in our cohort, supporting the presence of hepatic conditions frequent in older adults (e.g., hepatitis and bile duct obstructions)²⁸. Alterations in these parameters have also been associated with higher incidence rates of ischemic heart disease and other cardiovascular conditions, supporting these markers as useful for managing and predicting cardiovascular risk in the general population²⁹. A reduction in TRF levels was observed in 65% of the NCS pilot participants. This condition is commonly seen in ageing and is linked to liver dysfunction, iron disorders, and chronic inflammation. These issues tend to be more pronounced in older individuals and contribute to a range of health problems, including cardiovascular disease and cancer³⁰. Finally, a chronic inflammatory profile, represented by alteration of FBG, DD, and CRP inflammation markers, was clearly identified in our cohort. This enhanced inflammatory state, often referred to as "inflammaging", is associated with various age-related conditions, including cardiovascular diseases^{31–33}.

Validated in large cohort studies and longitudinal follow-ups, these biomarkers can help construct biological profiles that delineate ageing trajectories and identify health risk profiles. Studies such as the "Baltimore Longitudinal Study of Aging" (BLSA), which has been ongoing since 1958³⁴, and the "Invecchiare in Chianti" (InCHIANTI)³⁵, have extensively investigated a wide array of biomarkers related to cardiovascular, inflammatory, and metabolic conditions that provide insights into the ageing process and disease progression. For example, elevated levels of inflammatory markers like CRP and metabolic markers like HbA1c have been consistently associated with poor health outcomes and increased mortality in ageing populations^{36,37}. Similarly, the Italian cohort study Moli-SANI highlighted their importance in predicting cardiovascular diseases and diabetes³⁸.

Acknowledging that variations in biomarkers might be related to both diseases and/or ageing, we analyzed their distribution in relationship to the most prevalent chronic conditions³⁹. Unexpectedly, the biomarkers found significantly associated with CVD in our cohort - PLT, MONO, ALP, and ALB - differ from those identified in other Italian studies. In the Moli-SANI, CRP, fibrinogen, and glucose levels were the most deregulated markers²⁸. This discrepancy is possibly due to the low sample size of our study or the inclusion of a different representation of cardiovascular conditions. Conversely, biomarkers associated with diabetes were in line with other Italian studies like the "Progetto CUORE", reporting elevated HbA1c, WBC, TG, and B1, alongside concurrent reductions in HDL and LDL levels⁴¹. These alterations reflect diabetes-linked metabolic dysregulations, where HbA1c is a critical marker for long-term glucose control⁴².

To meet the primary aim of the NCS project, we investigated biomarker variation during aging. By comparing participants over and under 65 years, we identified a group of biomarkers associated with inflammation, metabolism, liver, and kidney function. Ageing is a multifaceted process that impairs various physiological systems. Kidney function naturally declines with age, primarily evidenced by a decrease in the eGFR, as reflected by elevated CR and BUN^{43,44}. DD and CysC, which are known to increase with age and are linked to cardiovascular risk^{45,46}, showed alterations in our cohort. Additionally, increased HbA1c, which is reported to rise with age even in the absence of diabetes or other metabolic diseases⁴⁷, was observed.

Overall, these preliminary results reveal a pattern typical of ageing that could form the basis for developing a comprehensive panel of biomarkers. Such a panel could predict biological ageing, thereby facilitating preventive and personalized interventions⁵. By integrating biomarker discovery through omics analyses, we can expand and refine this panel, making it more precise for identifying subclinical deficits and uncovering new therapeutic targets.

In conclusion, the preliminary findings of NCS pilot gave researchers essential pieces of information: the risk of selection bias due to volunteer participation must be mitigated by adopting a strategy that involves a random sample of the population; the analysis of the panel of blood biomarkers is reassuring, demonstrating sufficient variability across participants to effectively investigate the ageing trajectories; and the Novara population exhibited a high level of participation and involvement, which is crucial for the success of population studies like the NCS.

Declarations

Funding

This study was partially funded by the Italian Ministry of University and Research (MUR) program "Departments of Excellence 2023-2027," AGING Project – Department of Translational Medicine, Università del Piemonte Orientale, and is part of the AGE-IT project, which has received funding from the MUR – M4C2 1.3 of PNRR under grant agreement no. PE0000015.

Acknowledgements

We would like to express our gratitude to the Clinical Chemistry Laboratory of Maggiore della Carità University Hospital (Novara, Italy) for their invaluable support in the blood sample analysis. Furthermore, we thank Dr. Tiziana Cena (Unit of Epidemiology, Local Health Unit Vercelli – Italy) and Dr. Donatella Tiberti (SEREMI, Piedmont Region, Italy) for the provision and analysis of PASSI data.

Declaration of Conflicting Interests

We disclose the following potential conflicts of interest: Professor Daniela Capello, the study supervisor, is the scientific director of UPO Biobank (Center for Autoimmune and Allergic Diseases, Novara, Italy),

which supports this research and collects the biological samples. Additionally, several collaborators involved in this study hold positions within the Biobank. Nonetheless, we affirm that comprehensive measures were implemented to maintain objectivity and uphold scientific integrity.

Data Availability

The datasets used and analyzed during the current study were extracted from the REDCap database, which contains data collected as part of the Novara Cohort Study. All participants in this study provided informed consent that specifically authorized the analysis of their data in aggregated form and allowed for the sharing of these data with other research institutions, subject to ethical approval. Due to the sensitive nature of the information and the privacy agreements in place, the raw data are not publicly available. However, the datasets can be made available to other researchers upon reasonable request. Interested researchers should contact the corresponding author with a clear rationale for their request. Data sharing will be subject to approval by the relevant ethics committees and will adhere to the terms of consent provided by the study participants.

References

1. World Health Organization. Ageing and Health. <https://www.who.int/news-room/fact-sheets/detail/ageing-and-health>
2. United Nations. *World Population Ageing 2019*. 2019.
3. The Lancet Healthy Longevity. Ageing populations: unaffordable demography. *Lancet Healthy Longev*. Dec 2022;3(12):e804. doi:10.1016/S2666-7568(22)00272-0
4. Choi M, Sempungu JK, Lee EH, Lee YH. Living longer but in poor health: healthcare system responses to ageing populations in industrialised countries based on the Findings from the Global Burden of Disease Study 2019. *BMC Public Health*. Feb 22 2024;24(1):576. doi:10.1186/s12889-024-18049-0
5. Moqri M, Herzog C, Poganik JR, et al. Biomarkers of aging for the identification and evaluation of longevity interventions. *Cell*. Aug 31 2023;186(18):3758-3775. doi:10.1016/j.cell.2023.08.003
6. Li Y, Tian X, Luo J, Bao T, Wang S, Wu X. Molecular mechanisms of aging and anti-aging strategies. *Cell Commun Signal*. May 24 2024;22(1):285. doi:10.1186/s12964-024-01663-1
7. Khalatbari-Soltani S, Si Y, Dominguez M, Scott T, Blyth FM. Worldwide cohort studies to support healthy ageing research: data availabilities and gaps. *Ageing Res Rev*. Apr 2024;96:102277. doi:10.1016/j.arr.2024.102277
8. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr*. Sep 2006;9(6):755-62. doi:10.1079/phn2005898
9. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. May 1989;28(2):193-213. doi:10.1016/0165-1781(89)90047-4

10. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*. Dec 1991;14(6):540-5. doi:10.1093/sleep/14.6.540
11. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. Dec 2002;5(6B):1113-24. doi:10.1079/PHN2002394
12. Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess*. Dec 1996;67(3):588-97. doi:10.1207/s15327752jpa6703_13
13. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol*. Dec 1988;56(6):893-7. doi:10.1037//0022-006x.56.6.893
14. Feeney JA, Ryan SM. Attachment style and affect regulation: relationships with health behavior and family experiences of illness in a student sample. *Health Psychol*. Jul 1994;13(4):334-45. doi:10.1037//0278-6133.13.4.334
15. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. Aug 30 2005;173(5):489-95. doi:10.1503/cmaj.050051
16. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. Apr 2005;53(4):695-9. doi:10.1111/j.1532-5415.2005.53221.x
17. Nightingale CJ, Mitchell SN, Butterfield SA. Validation of the Timed Up and Go Test for Assessing Balance Variables in Adults Aged 65 and Older. *J Aging Phys Act*. Apr 1 2019;27(2):230-233. doi:10.1123/japa.2018-0049
18. Kammin EJ. The 6-Minute Walk Test: Indications and Guidelines for Use in Outpatient Practices. *J Nurse Pract*. Jun 2022;18(6):608-610. doi:10.1016/j.nurpra.2022.04.013
19. Western MJ, Malkowski OS. Associations of the Short Physical Performance Battery (SPPB) with Adverse Health Outcomes in Older Adults: A 14-Year Follow-Up from the English Longitudinal Study of Ageing (ELSA). *Int J Environ Res Public Health*. Dec 6 2022;19(23)doi:10.3390/ijerph192316319
20. Istituto Superiore di Sanità. La sorveglianza Passi d'Argento. <https://www.epicentro.iss.it/passi-argento/info/info>
21. Popovic, Heucker. Study Bias. StatPearls Publishing; 2023.
22. van Alten S, Domingue BW, Faul J, Galama T, Marees AT. Reweighting UK Biobank corrects for pervasive selection bias due to volunteering. *Int J Epidemiol*. Apr 11 2024;53(3)doi:10.1093/ije/dyae054
23. Wilkins CH. Effective Engagement Requires Trust and Being Trustworthy. *Med Care*. Oct 2018;56 Suppl 10 Suppl 1(10 Suppl 1):S6-S8. doi:10.1097/MLR.0000000000000953
24. Ritt M, Jager J, Ritt JI, Sieber CC, Gassmann KG. Operationalizing a frailty index using routine blood and urine tests. *Clin Interv Aging*. 2017;12:1029-1040. doi:10.2147/CIA.S131987
25. Blodgett JM, Theou O, Howlett SE, Rockwood K. A frailty index from common clinical and laboratory tests predicts increased risk of death across the life course. *Geroscience*. Aug 2017;39(4):447-455.

doi:10.1007/s11357-017-9993-7

26. Hao Q, Sun X, Yang M, Dong B, Dong B, Wei Y. Prediction of mortality in Chinese very old people through the frailty index based on routine laboratory data. *Sci Rep*. Jan 18 2019;9(1):221. doi:10.1038/s41598-018-36569-9
27. Jung E, Kong SY, Ro YS, Ryu HH, Shin SD. Serum Cholesterol Levels and Risk of Cardiovascular Death: A Systematic Review and a Dose-Response Meta-Analysis of Prospective Cohort Studies. *Int J Environ Res Public Health*. Jul 6 2022;19(14)doi:10.3390/ijerph19148272
28. Guerra Ruiz AR, Crespo J, Lopez Martinez RM, et al. Measurement and clinical usefulness of bilirubin in liver disease. *Adv Lab Med*. Aug 2021;2(3):352-372. doi:10.1515/almed-2021-0047
29. Katzke V, Johnson T, Sookthai D, Hüsing A, Kühn T, Kaaks R. Circulating liver enzymes and risks of chronic diseases and mortality in the prospective EPIC-Heidelberg case-cohort study. *BMJ Open*. 2020;10(3):e033532. doi:10.1136/bmjopen-2019-033532
30. Tian Y, Tian Y, Yuan Z, et al. Iron Metabolism in Aging and Age-Related Diseases. *Int J Mol Sci*. Mar 25 2022;23(7)doi:10.3390/ijms23073612
31. Stevenson AJ, McCartney DL, Harris SE, et al. Trajectories of inflammatory biomarkers over the eighth decade and their associations with immune cell profiles and epigenetic ageing. *Clinical Epigenetics*. 2018;10(1)doi:10.1186/s13148-018-0585-x
32. Meier HCS, Mitchell C, Karadimas T, Faul JD. Systemic inflammation and biological aging in the Health and Retirement Study. *GeroScience*. 2023;45(6):3257-3265. doi:10.1007/s11357-023-00880-9
33. Saavedra D, Añé-Kourí AL, Barzilai N, et al. Aging and chronic inflammation: highlights from a multidisciplinary workshop. *Immunity & Ageing*. 2023;20(1)doi:10.1186/s12979-023-00352-w
34. Shock NW, Greulich RC, Costa PT, Jr., et al. Normal Human Aging: The Baltimore Longitudinal Study on Aging. *NIH Publication*. 1984;doi:10.13016/SCLW-ICCQ
35. Ferrucci L, Bandinelli S, Benvenuti E, et al. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc*. Dec 2000;48(12):1618-25. doi:10.1111/j.1532-5415.2000.tb03873.x
36. Ferrucci L. The Baltimore Longitudinal Study of Aging (BLSA): A 50-Year-Long Journey and Plans for the Future. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*. 2008;63(12):1416-1419. doi:10.1093/gerona/63.12.1416
37. National Institute of Health. InCHIANTI Study. <https://www.nia.nih.gov/inchianti-study>
38. Iacoviello L, Bonanni A, Costanzo S, et al. The Moli-Sani Project, a randomized, prospective cohort study in the Molise region in Italy; design, rationale and objectives. *Italian Journal of Public Health*. 2007;4(2)doi:10.2427/5886
39. World Health Organization. Noncommunicable diseases. <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>

40. Bonaccio M, Di Castelnuovo A, Pounis G, et al. High adherence to the Mediterranean diet is associated with cardiovascular protection in higher but not in lower socioeconomic groups: prospective findings from the Moli-sani study. *International Journal of Epidemiology*. 2017;46(5):1478-1487. doi:10.1093/ije/dyx145
41. Istituto Superiore di Sanità. Il progetto Cuore.
42. Dorcely B, Katz K, Jagannathan R, et al. Novel biomarkers for prediabetes, diabetes, and associated complications. *Diabetes Metab Syndr Obes*. 2017;10:345-361. doi:10.2147/DMSO.S100074
43. Zhang Y, Yu C, Li X. Kidney Aging and Chronic Kidney Disease. *International Journal of Molecular Sciences*. 2024;25(12):6585. doi:10.3390/ijms25126585
44. Noronha IL, Santa-Catharina GP, Andrade L, Coelho VA, Jacob-Filho W, Elias RM. Glomerular filtration in the aging population. *Front Med (Lausanne)*. 2022;9:769329. doi:10.3389/fmed.2022.769329
45. McDermott MM, Liu K, Green D, et al. Changes in D-dimer and inflammatory biomarkers before ischemic events in patients with peripheral artery disease: The BRAVO Study. *Vasc Med*. Feb 2016;21(1):12-20. doi:10.1177/1358863X15617541
46. West M, Kirby A, Stewart RA, et al. Circulating Cystatin C Is an Independent Risk Marker for Cardiovascular Outcomes, Development of Renal Impairment, and Long-Term Mortality in Patients With Stable Coronary Heart Disease: The LIPID Study. *Journal of the American Heart Association*. 2022;11(5)doi:10.1161/jaha.121.020745
47. Masuch A, Friedrich N, Roth J, Nauck M, Müller UA, Petersmann A. Preventing misdiagnosis of diabetes in the elderly: age-dependent HbA1c reference intervals derived from two population-based study cohorts. *BMC Endocrine Disorders*. 2019;19(1)doi:10.1186/s12902-019-0338-7

Figures

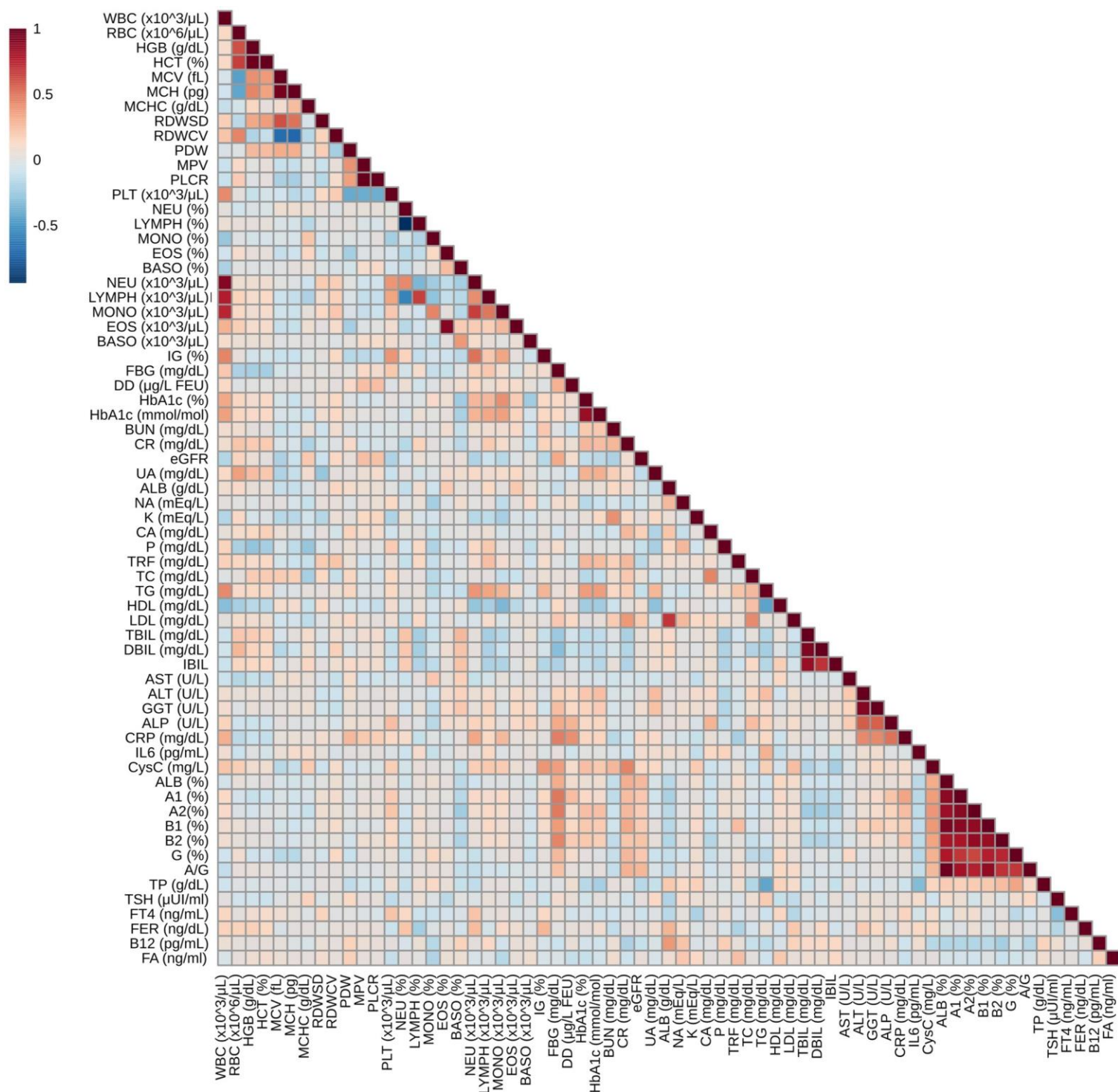


Figure 1

Heatmap of the 67 blood parameters based on Pearson correlation.

The graph visually represents the relationships between each pair of variables. The strength of each correlation is indicated by the Pearson correlation coefficient. Blue values denote negative correlations, while red values signify positive correlations. Lighter colors represent weaker correlations.

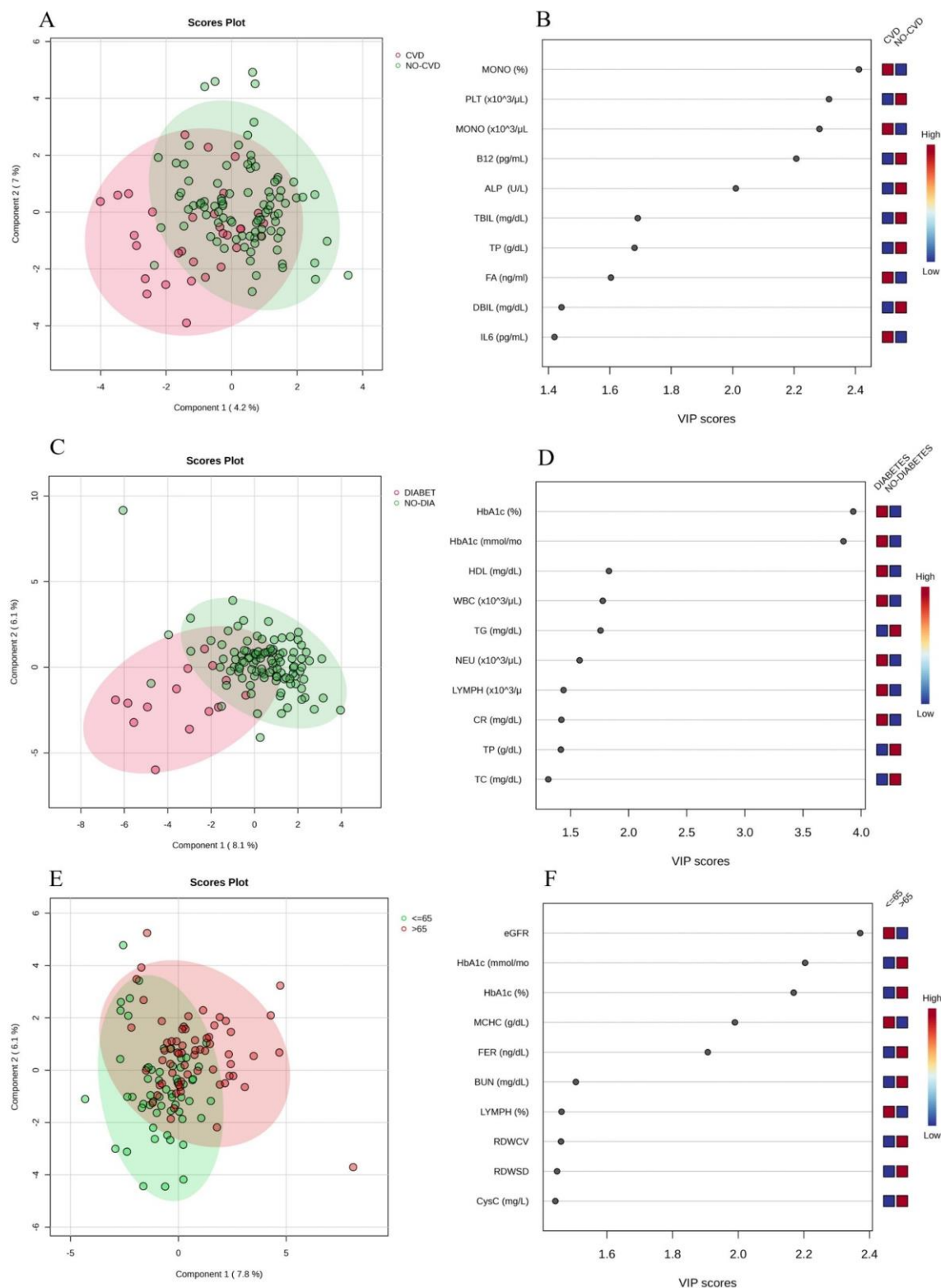


Figure 2

PLS-DA analysis of blood biomarker distribution in NCS pilot participants based on diseases and age

The scatter plot visually represents the separation of subjects based on the variables that distinguish them: the CVD group (in red) from the NO-CVD group (in green) **(A)**, the group of diabetic (in red) from the group of non-diabetic subjects (in green) **(C)**, and the group of over 65 years old (in red) from the

group of under 65 years old (in green) individuals **(E)**. Each data point corresponds to a participant, and the placement of these points shows their projection onto the discriminant components. Ordered list included, showing analytes with higher discriminative power based on variable importance in projection (VIP) scores for distinguishing individuals with and without CVD **(B)**, with and without diabetes **(D)**, and under and over 65 years old **(F)**. The classification achieves statistical significance for VIP scores >1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ♦ [SupplementarymaterialSR1.docx](#)

Project Report

UPO Biobank: The Challenge of Integrating Biobanking into the Academic Environment to Support Translational Research

Valentina Bettio ^{1,2,*}, Eleonora Mazzucco ^{1,2}, Chiara Aleni ³, Silvia Cracas ², Carmela Rinaldi ^{2,4}, Annamaria Antona ², Marco Varalda ², Jacopo Venetucci ², Daniela Ferrante ², Antonio Rimedio ⁵, and Daniela Capello ^{1,2}

¹ UPO Biobank, University of Piemonte Orientale, 28100 Novara, Italy

² Department of Translational Medicine, Center of Excellence in Aging Sciences, University of Piemonte Orientale, 28100 Novara, Italy

³ Department of Sustainable Development and Ecological Transition, University of Piemonte Orientale, 13100 Vercelli, Italy

⁴ Learning and Research Area, A.O.U. Maggiore della Carità, 28100 Novara, Italy

⁵ Ethics Committee of the University “Hospital Major of Charity” in Novara, Local Health Authorities Biella, 28100 Novara, Italy

* Correspondence: valentina.bettio@med.uniupo.it

Abstract: Biobanks are driving motors of precision and personalized medicine by providing high-quality biological material/data through the standardization and harmonization of their collection, preservation, and distribution. UPO Biobank was established in 2020 as an institutional, disease, and population biobank within the University of Piemonte Orientale (UPO) for the promotion and support of high-quality, multidisciplinary studies. UPO Biobank collaborates with UPO researchers, sustaining academic translational research, and supports the Novara Cohort Study, a longitudinal cohort study involving the population in the Novara area that will collect data and biological specimens that will be available for epidemiological, public health, and biological studies on aging. UPO Biobank has been developed by implementing the quality standards for the field and the ethical and legal issues and normative about privacy protection, data collection, and sharing. As a member of the “Biobanking and Biomolecular Resources Research Infrastructure” (BBMRI) network, UPO Biobank aims to expand its activity worldwide and launch cooperation with new national and international partners and researchers. The objective of this manuscript is to report an institutional and operational experience through the description of the technical and procedural solutions and ethical and scientific implications associated with the establishment of this university research biobank.

Keywords: biobank; disease biobank; population biobank; precision medicine; translational research; sample storage; data storage; sample management



Citation: Bettio, V.; Mazzucco, E.; Aleni, C.; Cracas, S.; Rinaldi, C.; Antona, A.; Varalda, M.; Venetucci, J.; Ferrante, D.; Rimedio, A.; et al. UPO Biobank: The Challenge of Integrating Biobanking into the Academic Environment to Support Translational Research. *J. Pers. Med.* **2023**, *13*, 911. <https://doi.org/10.3390/jpm13060911>

Academic Editor: Jivko V. Stoyanov

Received: 5 April 2023

Revised: 18 May 2023

Accepted: 26 May 2023

Published: 29 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Medicine is increasingly refining its ability to provide “custom-made” responses to patients and society. This personalized approach, based on the concept of the uniqueness of the person, enables the development of precision medicine, which helps to optimize prevention strategies and the most suitable therapies for each individual [1–4]. This paradigmatic shift in medicine is supported by the improvements in molecular investigations and bioinformatics, which allow the integrated analysis of a multiplicity of biological data with personal information (health, lifestyle, habits, socioeconomic, demographic, etc.), but imposes, at the same time, the need for a critical mass of biological samples and information collected according to the scientific criteria and high-quality standards [5–7]. Research biobanks have been created to meet these needs and, from the first appearance of the term biobank in a scientific report in 1996 [8], their diffusion and development have soared all over the world [9–16]. Biobanks can be properly defined as legal entities or part of a legal

entity that performs, in a standardized way, the acquisition, storage, and distribution of high-quality biological samples and associated data for research purposes [17–20]. Currently, there are hundreds of biobanks worldwide that range from small, predominantly university-based collections, to large, government-supported resources, whose activity, by supporting translational research, is focused on the public interest and aims to provide a public benefit for future generations [6,7,19–28].

UPO Biobank is the institutional research biobank of the University of Piemonte Orientale (UPO), housed at the Applied Research Centre Ipazia in Novara and integrated with the Regional Research Infrastructure Center of Autoimmune and Allergic Diseases (CAAD).

UPO Biobank came into operation in April 2020, during the first outbreak of the COVID-19 pandemic, to meet the need for a systematic and organized collection of samples to sustain research focused on preventive, diagnostic, prognostic, and therapeutic strategies [29–31]. In June 2020, once the first COVID-19 emergency was over in Italy [32,33], UPO Biobank was established as a disease- and population-oriented academic research biobank, with a focus on aging research. The UPO Biobank rules and code of ethics have been approved by the Ethics Committee and UPO authorities.

Today, the systematic collection of samples is well underway, in collaboration with local healthcare services, hospitals, and university research groups. In two years of activity, UPO Biobank has collected biological samples and data from more than 1100 subjects, stored up to 35,000 aliquots of human biological samples at +4 °C, −80 °C, and liquid nitrogen, and is emerging as a powerful infrastructure for UPO researchers. Indeed, UPO Biobank provides a rich source of biological materials (i.e., blood and derivatives, saliva, urine, and stool) and the associated data derived from the general population, COVID-19, kidney diseases, diabetes, hematological malignancies, neurological disorders, and autoimmune diseases.

The objective of this manuscript is to report an institutional and operational experience, describing the establishment of a university research biobank with the following purposes: (i) promoting the institution's quality scientific research with respect for all the parties involved; (ii) supporting cohort studies aimed at promoting and improving health in the reference area; (iii) endorsing public engagement and scientific citizenship actions; and (iv) exploiting samples and data, making them available to the whole scientific community.

2. Methods

2.1. UPO Biobank Operational Standards

All UPO activities are carried out in accordance with the Ethics Code, a regulation approved by Ethics Committee and UPO Authorities.

High-quality biological material and data are maintained and managed by applying standardized procedures (SOPs) and a quality management system, according to the ISO20387:2019 international standard, developed by the International Organization for Standardization (ISO), a worldwide federation of national standards bodies that work on preparing International Standards through ISO technical committees (www.iso.org/, accessed on 22 November 2022). ISO20387:2019, In particular, ISO20387:2019 specifies the general requirements for biobanking and lists the requirements that biobanks must fulfill to demonstrate their competence in the different aspects of the biobanking operations, as well as their ability to provide high-quality biological samples and associated data for research applications.

2.2. Ethical Statement

UPO Biobank code of ethics, regulation, and informed consent have been evaluated and approved by the Ethics Committee of the Ospedale Maggiore della Carità in Novara and by the University of Piemonte Orientale Data Protection Officer (DPO). Studies supported by UPO Biobank have been approved by the competent ethics committees. The

prerequisite for enrolment in these studies, as well as the aims and purposes of UPO Biobank, were depicted in the written informed consent.

2.3. Sample Management

The human biological samples stored in UPO Biobank are collected, registered, manipulated, and managed following SOPs. Each step of the biobanking process (i.e., sample collection, sample manipulation, sample aliquoting, temporary and long-term storage, release, and transport conditions) is documented in the UPO Biobank SOPs and quality manual, which is available to researchers upon request (UPO_biobank@uniupo.it).

The traceability of biological samples and associated data are assured by an unambiguous, numerical donor identification code and an acronym-specific sample identifier system. Each sample is linked to the associated dataset, whose minimal content is described within the manuscript.

Sample types include whole blood, buffy coat, plasma, serum, peripheral blood mononuclear cells, saliva, urine, and stool. Samples are stored based on the optimal conditions reported in literature and international standards and guidelines at different storage temperatures (i.e., +4 °C, −80 °C, and liquid nitrogen).

2.4. Quality Management

The UPO Biobank quality management system involves documented quality control (QC) policies of the biobanking process and SOPs that ensure the monitoring of collection, manipulation, aliquotation, preservation, and distribution of biological material and associated data. Non-conformities in any of these phases are recorded.

The hemolysis grade of plasma and serum samples is evaluated during the processing by assigning a growing value from 0 (none) to 3 (highly hemolyzed). UPO Biobank is setting up routine QC on plasma, serum, peripheral blood mononuclear cells (PBMC), and DNA. Indeed, to monitor the quality of the stored material over time, plasma, serum, PBMC, and genomic DNA are going to be processed and stored for this purpose following SOPs (QC samples). Selected analytes (e.g., reactive C protein, glutamic pyruvic transaminase, alanine transaminase, alkaline phosphatase, thyroid-stimulating hormone, transferrin, fibrinogen, D-dimer, and total proteins) are evaluated on QC plasma and serum samples at the time of collection and, subsequently, once a year. PBMC viability is evaluated before storage by Trypan Blue staining and automated cell counting (Luna II, Logos Biosystems) and recorded. A cell viability control is going to be carried out annually on QC PBMC samples. DNA concentration and purity are determined by measuring the absorbance at 260 and 280 nm in a spectrophotometer (NanoDrop™).

The correct allocation of the aliquots in the cryogenic devices is checked biannually. The UPO Biobank Quality Manager simulates the research and retrieval of 10 to 30 different, random aliquots of biological material by consulting the REDCap database and checking their effective positioning in the devices. In the event of discordances, the malposition is reported as a non-conformity and treated accordingly. Similarly, the associated data are checked biannually. The UPO Biobank Data Manager simulates the research and retrieval of the associated data of 10 to 30 different subjects on the REDCap database and checks their consistency, reporting, and treating the non-conformity derived by a possible discordance.

2.5. Data Analysis

All UPO Biobank data are stored in the REDCap database (REDCap, Vanderbilt University). Data presented in this paper were derived from the REDCap database and analyzed using the GraphPad Prism software, version 8 (GraphPad Software Inc., San Diego, CA, USA). Data were presented as numbers and percentages.

3. Results

3.1. UPO Biobank Finalities

UPO Biobank has been established as a multidisciplinary research biobank with both a population- and disease-oriented commitment, with the aim of promoting studies finalized at exploiting knowledge on human health and encouraging multidisciplinary scientific research on aging. The research areas and objectives of the biobank are clearly outlined in the biobank regulations and, for a consent to be clearly informed, in the UPO Biobank broad informed consent.

As a disease-oriented biobank, UPO Biobank promotes research on human diseases to improve prevention, diagnosis, and therapy within specific areas of interest, such as infection, autoimmune disorders, and high-impact, chronic, age-associated diseases, including cardiovascular, metabolic, neurodegenerative, and neoplastic diseases. Patients participating in disease-specific project biobanking are also asked to participate in the broader aims of the biobank by providing a specific broad consent (discussed later in this paper).

Based on this biobank initiative, disease-specific biobanking studies have already been published (Table 1) [34–40], and all the invited patients participating in these studies also adhered to the broader aims of the biobank by providing specific consent.

Table 1. Main UPO Biobank biobanking projects.

Study	Description	Collection Period	Number of Participants	Publications
UPO-COVID-19	Collection of blood from COVID-19 patients during the very first outspread of the disease in Italy (April–June 2020).	2020	106	/
UnIRSa Cohort Study Unveiling the Immune Response to SARS-CoV-2 Infection	Evaluation of the immune response, during the time, to SARS-CoV-2 epitopes in patients recovered from COVID-19 and the efficacy of specific anti-SARS-CoV-2 antibodies as neutralizing agents.	2020–2021	100	Griffante, G. et al., 2021 [36]
ddPCR OnCOVID Comparative Analysis of SARS-CoV-2 Molecular Techniques on Different Biological Samples in a Cohort of Patients Undergoing Anticancer Therapy	Investigation of droplet digital PCR (ddPCR) and neutralization assay (NTA) for the management of SARS-CoV-2-infected onco-hematologic patients (i.e., to detect the neutralizing antibodies).	2021	33	Borgogna, C. et al., 2022 [34]
A.O.U. ddPCR SARS-CoV-2 Viral Surveillance on the Medical Staff of the A.O.U. “Maggiore della Carità” by Droplet Digital PCR Analysis Performed on Saliva Samples	Comparison between oropharyngeal swab and saliva samples for the detection of SARS-CoV-2 infection by ddPCR, with the purpose to better define a sanitary surveillance strategy for the healthcare personnel of the A.O.U. “Maggiore della Carità”.	2021	18	/

Table 1. Cont.

Study	Description	Collection Period	Number of Participants	Publications
ema-NTA Immunological Surveillance Protocol to Assess the Effectiveness and Duration of Post-infection Immunity from SARS-CoV-2 in a Cohort of Hematological Patients	Analysis of the humoral immune response in SARS-CoV-2-infected hematological patients, who have had and got over COVID-19 disease with a neutralization assay.	2021–2022	177	Borgogna, C. et al., 2022 [34] Borgogna, C. et al., 2022 [35]
BioMAge Pilot Study for the Identification of Biomarkers Signatures of Aging and Longevity	Identification of aging biomarkers that will help to define the individual rate of aging, the risk of illness and death, and the impact of longevity interventions.	2020-ongoing	193	Bettio, V. et al., 2023 [38]
DM-PREVENT Novel Intestinal Microbiota-based Medicine for Preventing Type 2 Diabetes Mellitus	Assessment of the effects on insulin sensitivity of the probiotic containing <i>Intestinimonas butyriciproducens</i> in adults with prediabetes.	2021-ongoing	28	/
TED Prevention of Falls in the Elderly Using the Monitoring System TED	Assessment of the efficacy of the wristband TED in the identification of falling-risk patients affected by Parkinson's Disease.	2020-ongoing	20	Campani, D. et al., 2022 [37]
NO-MORE-COVID-19 Early Diagnosis of Comorbidity and Assessment of the Effective Immunization in COVID-19 Patients	Assessment of respiratory, radiological, motor, and psychological sequelae in COVID-19 patients after 1 year from hospital discharge.	2021-ongoing	301	Bellan, M. et al., 2022 [40]
KETOMI VLCKD (very-low-calorie ketogenic diet) in patients with type 2 diabetes and non-alcoholic fatty liver steatosis	Investigation of the complex interplay between inflammation, hormones, microbiota composition and functions, and messengers (e.g., extracellular vesicles and metabolites) to give tailored indications in the clinical management of type 2 diabetes and non-alcoholic fatty liver steatosis.	2022-ongoing	3	/
KT-UPO-B Creation of a Biobank for the research of biomarkers related to immunological and non-immunological dysfunction of the graft in patients with renal transplant	Collection of biological samples from a large cohort of patients with a renal transplant, with a long systematically documented clinical–laboratory follow-up for the identification and validation of new biomarkers related to the main complications of renal transplantation.	2023-ongoing	2	/
NCS Novara Cohort Study	Longitudinal cohort study aimed at the identification of biological, social, and environmental determinants associated with the different trajectories of aging in the Novara area (Italy).	2022-ongoing	56	/

Table 1. Cont.

Study	Description	Collection Period	Number of Participants	Publications
SIDERALE Susceptibility to infectious diseases in obese patients: an endocrinological, translational and sociological analysis	Finalized to demonstrate the association between oral and intestinal microbiota with the susceptibility to generic infections in obese patients.	2023	/	/
DELIVIDA Determination of plasmatic Vitamin D in elderly pulmonic or septic patients	Investigating the association between plasma vitamin D levels and the prognosis in elderly subjects hospitalized for either pneumonia of any origin or sepsis.	2023	/	/
OPTION Prospective cohort of patients affected by pulmonary hypertension: discovery of diagnostic and prognostic markers	Evaluating the role of the extracellular vesicles in both pathogenesis and diagnosis of connective tissue-associated pulmonary hypertension.	2023	22	/

As a population-oriented biobank, UPO Biobank supports prospective and cross-sectional epidemiological cohort studies involving citizens with specific characteristics or who are representative of a geographical area, designed to address both social and scientific unmet needs on human health. In particular, at present, UPO Biobank is engaged in the Novara Cohort Study (NCS), a longitudinal population study aimed at investigating the determinants of longevity and promoting healthy aging in the Novara area.

UPO Biobank is also a non-profit public academic institution and a “service unit” (BBMRI-ERIC:ID:IT_1611942116226242), whose mission is characterized by three main purposes: (1) to encourage and increase a collaborative network between universities, bodies, and associations dealing with public health, in particular hospitals and territorial competent local health services; (2) to encourage the training of university students, so that they are initiated into rigorous research on a scientific level and respectful of ethical principles; and (3) to promote a model of active scientific citizenship, by enhancing citizens’ involvement in public health research and social measures and by constructing communicative strategies able to promote the active, responsible and critical participation of citizens, local institutions and relevant stakeholders in the building of a shared scientific information and awareness.

3.2. The UPO Biobank Facility

UPO Biobank is an academic infrastructure consisting of three interconnected facilities: the consulting room, the processing laboratory, and the cryogenic room. Being located in the same building, the three facilities represent a core, integrated biobank structure that supports all of the biobanking-related activities, from the collection of data and biological samples to their manipulation, preservation, and release.

The consulting room is a key facility to conduct population- and disease-oriented studies supported and coordinated by UPO Biobank, as well as a valuable service for studies carried out by independent research groups and destined for biobanking. The room is equipped for the evaluation of functional and anthropometric parameters and for the collection of biological materials, providing both proper privacy and connected services (e.g., private and dedicated toilette). Moreover, an adjacent reserved space has been arranged to meet participants for questionnaire administration and data collection.

The processing laboratory is dedicated to sample manipulation, aliquoting, and analysis. Each operation is carried out following specific and detailed SOPs, and the tracking of

samples and aliquots is assured by a multiparametric codification via sample-associated codes and barcodes uploaded in the UPO Biobank database.

The management and traceability of the biobanked samples have proven to be demanding, with a real risk of mistakes and failure [41]. At the beginning of UPO Biobank activity, an in-house adapted system based on the REDCap database (REDCap, Vanderbilt University) was built to accommodate and track the storage of biological samples, as well as the pre-collection and post-storage operations (e.g., study management, informed consent, sample release, etc.). However, the growing demand for these activities prompted the acquisition of a commercial Laboratory Information Management System (LIMS) oriented and structured to answer to the unique needs of biobanking, which will be implemented in the next few months.

The cryogenic room was built following the specific indications for this kind of facility (i.e., ISO11827:2021). It houses 4 °C and −80 °C mechanical freezers and liquid nitrogen tanks connected to an automated system for nitrogen refill. The biobank can accommodate up to 200,000 samples at −80 °C, and up to 500,000 samples in liquid nitrogen.

Since UPO Biobank was implemented with the aim of supporting large population studies, storage capacity was a key issue from the beginning of the UPO Biobank activity planning. In this perspective, the cryogenic room has been equipped with nitrogen tanks fitted to contain straws, doubling their capacity with respect to the classical storage in cryoboxes. This implementation has been linked to an improvement in the processing of the samples by adopting a semi-automated aliquoting system for fluid samples (i.e., plasma and serum), consisting of two MAPI2 machines (Cryo Bio System, L'Aigle, France) that fill, seal, and label the straws.

The samples, collected following specific SOPs, typically include whole blood and its fractions, extracted genomic DNA, whole cell RNA, extracellular vesicles, urine, saliva, and stool. The standardization of the processes has been further implemented by adopting a programmed abatement system for live-cell cryopreservation (Kryo 560-16, Planer), a key instrument for live-cell biobanking that is also suitable for the preservation of cells for cell-based therapy [42–45].

Access to the cryogenic room is only allowed for authorized personnel through electronic badges and a video surveillance system. The management of the bank is supported by a centralized control system capable of monitoring and recording all the operating parameters (the percentage of ambient oxygen, the liquid nitrogen supply, and the temperature of the cryo-containers) for 24/24 h and 7/7 days. Critical alarms are sent via SMS, voice call, or email to operators for intervention in the event of an emergency. The “life” of each biological sample, from acceptance to analysis, including storage positions, movements, and possible criticalities, is traced by the tracking system and transferred to LIMS.

3.3. Building an Efficient Biobank: Networking, Quality Management System, and Certification

The “Biobanking and Biomolecular Resources Research Infrastructure—European Research Infrastructure Consortium” (BBMRI-ERIC) is a pan-European non-profit network of biobanks aimed to improve the accessibility and interoperability of the biobanks of the network as well as to prompt and support the harmonization of the different aspects of the biobanking process [46–48]. UPO Biobank joined the national node of the network (BBMRI.it) in February 2021, heading from the beginning toward both an efficient standardization of activity and an incisive interaction between national and European partners. BBMRI-ERIC eases the interaction between biobanks and biobank users by enabling access to the collection of partner biobanks, as well as by supplying expertise and services (i.e., ELSI common service and IT common service). The effective harmonization and interaction among biobanks and between biobanks and final users is a key goal to achieve in the biobanking field, and BBMRI-ERIC, also playing at a national level, is a major promoter of this implementation.

UPO Biobank also adopts strategies to enhance and maintain the quality of the biological samples, starting from their handling to their final preservation, as well as to ensure

data protection. Toward this aim, UPO Biobank runs a quality management system that is in agreement with national and international indications [34]. In particular, the quality management system of a biobank contains documented quality control policies and written standard operating procedures underlying the collection, manipulation, aliquotation, preservation, and distribution of biological material and associated data [49–51]. The whole biobanking process, from the acquisition to the storage and release of biological specimens and data, is based on Standard Operating Procedures (SOPs) and on the application of guidelines for the equipment used (e.g., sample processing, semi-automated aliquoting, databases, etc.). The UPO Biobank Quality Manager prepares, supervises, updates, and makes available the latest version of all documents, including instructions and authorizations for staff members, control system documents, and SOPs. Following joining BBMRI.it, UPO Biobank is about to complete the internal adaptation for the biobanking-specific ISO20387:2019 certification, which is expected to be achieved by mid-2023.

3.4. Sample Storage and Usage: Looking Forward to New Applications

The worldwide spread and growth of biobanks has brought to light the critical aspect of the cost–benefit balance between sample handling and preservation over time, which takes into account the fitness for purpose of the stored samples for future research applications, along with the burdensome and constantly growing employment of physical space and dedicated equipment. However, it is hardly reliable to be able to foresee every future request and application of the biobanked samples. An important aspect of a biobank's governance and framework is thus the definition of the sample types and the number of aliquots/samples to be collected and stored, taking into account the storage cost over time and the possible effective research applications of the collected samples and data. The key point is that it is not mandatory to collect a huge panel of biological materials but to be able to provide exhaustive documentation of the entire life cycle of the biobanked samples and data, from donor to release, ensuring a sufficient level of adequacy for research purposes. With a view of optimizing the availability of samples and their suitability for future omics investigations, UPO Biobank stores blood and plasma samples collected in the presence of EDTA, useful for proteomic, lipidomic, and genomic analysis [52–54], and in the presence of sodium citrate, useful for the downstream purification and analysis of extracellular vesicles (EVs) [55]. Likewise, urine is preserved in large aliquots and pellets, suitable for EV isolation and marker identification [56,57]. Finally, saliva collection optimized with Salivette®, a device that allows an easy and fast collection of saliva while avoiding spitting, can be successfully used for different downstream applications [58–60].

3.5. Access to UPO Biobank

Aiming for a participatory governance model, UPO Biobank encourages the direct interaction between the biobank and stakeholders with the purpose of facilitating useful communication to highlight their needs and expectations and to achieve a fruitful collaboration. To this end, on the website <https://biobank.uniupo.it/> (accessed on 24 November 2022), UPO Biobank provides an area dedicated to both citizens and researchers. On the dedicated pages, citizens can find information about the scientific, ethical, and social relevance of participation in biobank activities, the rights of the participants, and an application form to participate. The researcher-dedicated web site area reports all the relevant information about biobanking and how to access samples/data. An inquiry form that can be filled out online and directly sent to the biobank allows researchers to receive information about the following: (i) the availability of specific samples/data, and (ii) the application for a new biobanking project.

UPO Biobank's staff takes care of answering inquiries within 96 h after receipt and provides assistance for accessing the resources of the biobank or for a new project proposal. All requests are evaluated by the UPO Biobank Technical-Scientific Committee and approved or rejected according to the UPO Biobank Regulation and Ethics Code (which are

available online). In all cases, projects supported by UPO Biobank must be approved by the competent Ethics Committee.

The detailed procedures and documents for requesting information can be found on the biobank website (<https://biobank.uniupo.it/per-i-ricercatori/>, accessed on 24 November 2022). At the moment, the site is only in Italian, but an English version will be available soon.

3.6. Biobanking and Databanking

The true value of a biological sample is represented by the associated data, including information about the subject who provided the sample and the information obtained through the laboratory analysis of the biospecimens. Traditionally, such information is stored and managed separately from the samples, both for data size and for security, but with the concrete risk that with time, this information can be lost. Steps forward in data storage and management accessibility and security have allowed biobanks to store both samples and data with increasing benefits for the quality and usability of this wealth of information, moving away from the reductive concept of a biobank as a simple biorepository toward a biobank that manages and possibly generates data associated with samples [61,62].

UPO Biobank, in collaboration with the CAAD bioinformatics facility, is implementing the collection of multi-omics, imaging, or other biologic data, and is improving the databanking capacity and security by opting for cloud storage and backup of the data, in compliance with Article 32 and Article 89 of the General Data Protection Regulation (GDPR), and observing DPO guidelines, as well as the technical implementation and risk minimization measures resulted from the Data Protection Impact Assessment (DPIA).

3.7. An Institutional Biobank Supporting Translational Research and Population Studies

As a disease-oriented biobank, in addition to sustaining COVID-19 research, UPO Biobank is primarily finalized to promote and sustain research on high-impact, chronic diseases, including cardiovascular, metabolic, neurodegenerative, and autoimmune diseases. Researchers are supported by UPO Biobank's staff in the study design, in identifying the most suitable protocols for the collection and treatment of samples according to project-specific requirements, and in the presentation of research protocols to the Ethics Committee. Moreover, UPO Biobank facilitates the access of samples to the UPO core facility for laboratory investigations, including omics analysis.

From its birth in April 2020 until today, UPO Biobank has experienced a gradual increase in the number of supported projects and collected samples (Figure 1A). At present, UPO Biobank hosts samples and data from six concluded research projects and supports seven ongoing ones (Table 1).

At present, UPO Biobank hosts more than 30,000 aliquots of biological samples collected from about 1100 subjects, including whole blood, buffy coat, plasma, serum, peripheral blood mononuclear cells (PBMCs), urine, saliva, stool, and DNA, as shown in Figure 1B. A detailed overview of the samples and associated data actually stored in UPO Biobank are reported in Tables S1 and S2.

About 33% of the samples were collected as part of population studies, whereas nearly 67% of the samples were collected in longitudinal and cross-sectional disease-focused projects, including acute COVID-19 infection and long COVID-19 syndrome, chronic and acute kidney diseases, kidney transplant, and diabetes (Figure 1C).

The age median of the participants is 58 years old (Table S3), with a slightly higher adhesion from female participants with respect to male participants (51.1% vs. 48.9%, respectively, Figure 1D).

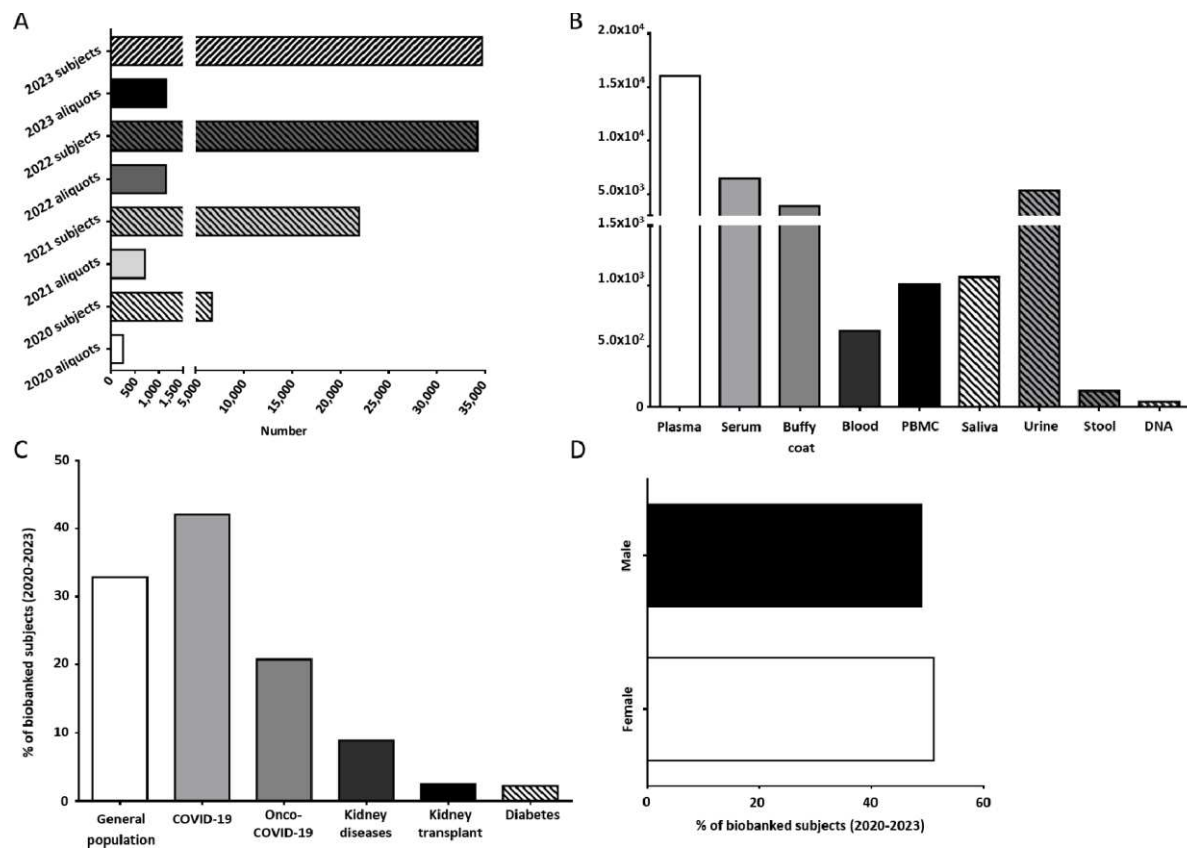


Figure 1. (A) Number of subjects and biological samples stored by UPO Biobank from April 2020. (B) Number of aliquots of the different biological samples stored in UPO Biobank until today. (C) Distribution of the biobanked subjects, highlighting the general population and specific disease-affected subjects. (D) Distribution (%) among female and male participants in UPO Biobank.

From the beginning of the activity, UPO Biobank efficiently supported translational research in UPO, with more than 600 samples released in the past year utilized for three COVID-19-related scientific publications, investigating the response to SARS-CoV-2 infection in the general population and in cancer patients [34–36,40], and other projects in progress.

As a population-oriented biobank, UPO Biobank is engaged in the NCS, a longitudinal population study that aims to define the molecular, physiological, and environmental determinants of the trajectories of aging in the area of Novara. The NCS will enroll 10,000 participants older than 35 years old, with a prospective follow-up every 5 years, and the collection of both biological specimens and personal data. For this study, blood, urine, and saliva collection, analysis, and storage were established, creating a multifaceted and constantly growing source of samples and associated data. The biological analysis will contribute to elucidating the molecular mechanism of aging and age-associated diseases. Longitudinal data analysis will also favor the identification of preventive intervention priorities in collaboration with local health authorities. Because of the extensive data and sample collection, the NCS and UPO Biobank will be uniquely rich resources for aging research for all scientific communities.

By supporting the NCS, UPO Biobank will become an integral part of the community, and, in turn, the Novara community will become an extended laboratory. Consequently, the Novara citizens will also find themselves as citizens of the global research community through international research projects.

3.8. The Key Role of the Governance

The legal and ethical aspects of biobanking are key matters in a biobank framework to optimize the use of biobank resources by the scientific community for quality scientific production while respecting the ethics and privacy of the participant. A good governance system based on the three essential pillars of transparency, accountability, and oversight is fundamental for increasing the legitimacy and social engagement of biobanks [63,64]. Particular attention is dedicated to data storage and sharing, with Article 40 of the GDPR encouraging the adoption of a code of conduct aimed at ensuring compliance with the legal requests regarding data processing and privacy [65]. UPO Biobank matched these requirements by adopting a governance system and operational procedures exhaustively described in the biobank regulation, available on the UPO Biobank website [66]. The UPO governance was structured to ensure the engagement of both the scientific community and the population. The Scientific Director (SD), the Strategic Oversight Committee (SOC), and the Technical-Scientific Committee (TSC) (Figure 2) represent the Scientific Board of UPO Biobank, and their role is to direct, lead, and sustain the biobank activity, ensuring the quality of research supported by the biobank's resources and the respect of the ethical requirements, working together with the competent Ethics Committee. The functioning of biobanking is guaranteed by a Technical Manager (TM), a Data Manager (DM), a Quality Manager (QM), and a Study Nurse (SN).

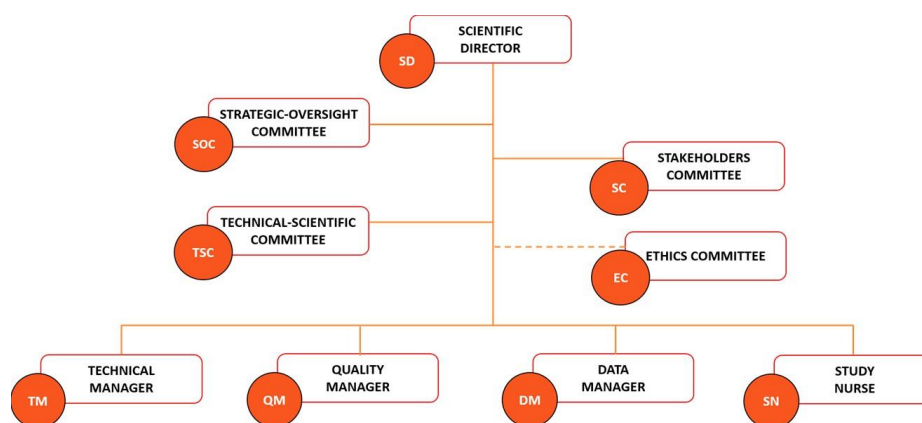


Figure 2. UPO Biobank governance chart.

Biobanks' activity should be founded on multifaceted perspectives that include both lay stakeholders (e.g., community members and organizations, policy-makers, and patient associations) and field experts (e.g., researchers, clinicians, and ethicists) [63,64,67]. The Stakeholder Committee is a pillar of the UPO Biobank governance chart (Figure 2) and code of conduct, with a scheduled annual meeting focused on the critical evaluation of UPO Biobank activities, aims, and future perspectives. The goal is to achieve constructive communication between UPO Biobank and citizens, ensuring the matching of the stakeholders' expectations and needs and an interdisciplinary approach to biobanking. These interdependent relationships ensure a deep understanding of the notion and impact of biobanking activity by the lay population, which aims to increase the general approval, trust, and active participation of citizens in future biobanking projects, as well as the safeguarding of the necessities and perspectives of potential biobank's users, avoiding the low utilization rates of the biobanked material [68,69]. A biobank acts, indeed, as a mediator between the several interests of researchers, clinicians, scientists, participants, and the general population, and must consider all viewpoints in order to make strategic decisions. This participatory governance aims to grant benefit to all the actors of the biobanking process, starting from the participants that entrust the biobank with their biological material and data, allowing new important scientific and health-related discoveries and advancements by the biobank's customers, which will benefit from a centralized, organized, and certified

source of information and material, ensuring in return a concrete and valuable benefit for the participants and the community in general.

3.9. Ethical, Legal, and Social Issues

The nature of data processing is a critical issue for the rights and freedoms of subjects, mainly for particular categories of data, such as genetic ones. On this topic, the GDPR, which was adopted by the European Union (EU) in 2016 and came into force in 2018, has proven to be challenging for the biobank community [70,71].

As a recently founded biobank, UPO Biobank immediately faced the integration of GDPR indications into its operational routine. In this regard, data security is a major concern regarding data sharing and usage for research purposes; therefore, before starting the biobanking activity, UPO Biobank carried out the DPIA for the assessment of the impact on data protection, as provided for by Articles 35 and 36 of the GDPR. Indeed, the DPIA is, for all intents and purposes, a mandatory tool to identify and minimize the risks that are inherent to data processing [72–74]. The DPIA outcome was constructively applied to improve the data management and security in the UPO Biobank, and it will be updated throughout the lifecycle of the biobank in order to ensure that data protection and privacy are considered and to promote the creation of solutions and compliance.

The GDPR imposes several obligations to scientists, particularly in relation to secondary research uses of personal data (i.e., data used for research studies other than the proposed research that enable biobanking). Biobanks, by implementing the GDPR, together with the technical measures that are necessary to safeguard the rights of data subjects during data processing, can guarantee a profitable use of data for research purposes [69,71,75], but they also face various challenges. The lack of supranational and national bodies with the competence and authority to set uniform and binding requirements causes a certain degree of uncertainty in all the actors involved (researchers, participants, and interested parties) and generates the need for regulations that are specifically dedicated to biobanking [69,71,72,75–77]. In Italy, the main reference points are the general authorizations issued by the Italian Data Protection Authority: Authorization no. 8/2016 on the processing of genetic data, and Authorization no. 9/2016 on the processing of personal data for scientific research purposes, but neither of the two authorizations contains provisions directly addressing biobanks [78,79].

Pending an ad hoc legislative act on biobanking, the adaptation for the biobanking-specific ISO20387:2019 certification and accreditation process provides the core elements and guarantees regarding the governance of UPO Biobank in view of GDPR, including conditions for processing personal data, data access models, oversight bodies, and data access and transfer agreements. Furthermore, the adoption of informed consent that clearly exemplifies the research purposes for which the biobanked samples/data and related information will be used, guarantees participants the freedom to decide how their samples will be used.

The social challenges raised by the activity of biobanks are manifold and include the need to adopt a communication strategy that must make use of a shared language, the knowledge and cultural effects resulting from results dissemination, and the cultural and social and not just scientific value of sustainability [80–83].

UPO Biobank aims to encourage and enhance scientific research by basing its activity on an inclusive model of the scientific community in which citizens, researchers, and institutions will actively participate. For this reason, biological specimens and associated information are recognized as public and institutional resources; the code of ethics and informed consent are deemed institutional documents; and the Stakeholders Committee is a pillar of the UPO Biobank organizational chart [66].

The UPO Biobank's code of ethics is inspired by the model of “participatory governance” that needs the involvement of citizens, who entrusted biological samples and associated data, researchers, and stakeholders, who support the biobank and have expectations from the biobank's activities. Starting from individual personal knowledge,

this model is based on the shared, intrinsic ability to understand and evaluate technical information, when adequately exposed, in order to participate in public and global decisions [77,84]. By adopting this organizational model, UPO Biobank will pursue the aim of achieving the virtuous circularity that transforms individual contributions into public benefit. The balancing of the potentially divergent needs and interests of these parties is certainly challenging, and the governance system must be proportionate and knowledgeable, especially regarding the risk associated with data sharing and use aimed at producing collective benefits [84,85].

3.10. From the Study-Specific Informed Consent toward a Model of Mixed Informed Consent

Starting from the study-specific informed consent, designed to ensure that the participants were deeply informed about the aims and the use of samples and data in the context of a specific and single research, biobanks moved to a new, adapted form of broad informed consent [86,87]. Indeed, although the study-specific consent clearly addresses most ethical issues, this type of consent is not suitable for the purposes of a biobank, which collects biological materials and personal data, especially for long-term future research, which is not clearly defined at present. Moreover, broad consent is less specific than consent for each use, but more narrow than open-ended permission without any limitations (i.e., “blanket” consent).

The broad informed consent approach aims to integrate the specific needs of biobanking while preserving the indisputable protection of the participants [87,88]. This model provides for the gathered general consent when the participant is enrolled and predisposes the future usage of the collected samples for new studies that fulfill the scope stated in the consent, avoiding the necessity to request consent again from the same participants [87–89].

The broad model based on general consent is still debated since it is hard to define a priori the future usage and application of the biological material and data collected, and, for consent to be informed, exactly this kind of knowledge is required [89–93]. However, for the purposes of conservation of biological samples, broad informed consent is indicated as appropriate both in the literature and in GDPR recital 33, although the references are fragmentary [90,94].

From these considerations, UPO Biobank and the territorially competent Ethics Committee (for the Ospedale Maggiore della Carità in Novara and the health Agencies of the surrounding area) felt that a “mixed” informed consent, which merges the specific and the broad ones, best suited the biobank aims, a perspective also shared by the Italian Node of BBMRI-ERIC [74]. Indeed, the nature of the consent given by the participants is mixed by nature, since the biobank’s purposes are general but not generic and thrown toward future biobank goals and applications, and in parallel, the information given to the participants about the manipulation and storage of samples and data, as well as their sharing with the scientific community, must be precise and rigorous. Mixed informed consent also presents advantages from an operational point of view. Indeed, by combining the specific research objectives and the broad biobanks’ purposes and aims, integrated with detailed information about personal data processing and protection, mixed informed consent provides a simplified tool intended to facilitate both the request of biobanking from clinicians and researchers and the reading and explanation of the information to the participants. Finally, the broad component of mixed informed consent is a key instrument for explaining and elucidating the general aims and areas of interest of UPO Biobank, ensuring a thorough understanding by the participants of the whole biobanking activity and its impacts. In order to ensure that this type of consent provides protection of autonomy and participant values, a strong ethical review of projects supported by the biobank and continuous communication activity is pursued.

3.11. The Interaction with Participants: A Matter of Trust

Although biobanks are widely recognized as a powerful and almost essential tool for biomedical research [19,22,95,96], their activity is not exclusively carried out in clinical and

research environments, but necessarily, it relies on the involvement of participants that provide biological samples and information that will sustain multiple research projects.

The unsuccessful enrolment of participants is an actual menace to a biobank's activity and "trust", in the view that to confide in biobanks and in those that oversee biobanking to protect participants' interests, is a matter of utmost importance in the field and remains, indeed, the core value in the relationship between a public biobank and participants [97–99]. A major concern for participants is the concept of "personality rights" in relation to how genetic information will be interpreted and used, and who should have access to it, because genomic information is detailed, identifiable in nature, and it affects both the person from whom the information was obtained and the related family members. These are the basic reasons why a biobank, and in particular a population biobank, must match the trust of citizens with the trustworthiness of its governance, as clearly stated by the Organization for Economic Co-Operation and Development (OECD) [100].

UPO Biobank, in conjunction with the launch of the NCS, undertook an intense public awareness campaign through the organization of and participation in public events, institutional stakeholders engagement, mainstream media dissemination, and training interventions among students. A recent survey investigating the level of knowledge and perception of biobanks in UPO students and personnel shows that potential participants were aware of the role that biobanks play in research and were eager to participate for the sake of furthering scientific research. Notably, the study highlights concerns about the confidentiality of the data along with the commercial use of the samples/data [39], encouraging UPO Biobank to disseminate clearer and detailed information about participant rights protection.

3.12. Deal with the Challenge: Limitations and Complications in Building an Institutional Biobank

The aspect of sustainability, defined as the maintenance over time of a biobank's operations and values, has always been a challenge [61,101]. In particular, economic sustainability is a key obstacle to the operability of biobanks over the years, and even though multiple strategies and tools for the cost recovery have been proposed [61,102–104], the costs related to the several operational aspects of biobanking exceed the cost-recovery ability of these facilities. On the other hand, biobanks are emerging as pillars for biomedical research and a priority for the European Community, a fact underlined by the integration of biobanks in different framework programs of Horizon 2020, Horizon 2022, and Piano Nazionale di Ripresa e Resilienza (PNRR) funded by Next Generation EU [105,106]. Biobanks' survival and growth, over time, need both researchers and institutional support. Researchers are called to contribute by designing research projects involving biobanking or using biobanked data/samples and by providing the institution with funding and qualified personnel. As an institutional biobank, UPO biobank is integrated into the academic environment, sharing the typical funding hindrances of this institution. As a non-profit reality, UPO Biobank is working on a cost-recovery plan that aims to cover the operational costs related to biological samples' management and storage, as well as structure and instrument maintenance. However, in the academic context, effective and long-term planning of their income is challenging, due to the irregular gain of funding. The full integration of biobanks in the academic research routine, foreseeing biobanking costs in grant applications, is the first step to achieve a structured cost recovery. For the future, a primary objective for UPO Biobank will be to cover at least part of the costs of maintenance through the acquisition of external resources. National and international academic collaborations and Horizon 2020 calls represent excellent opportunities, as do partnerships with biotech or pharma companies, always in compliance with the biobank code of ethics.

UPO Biobank infrastructural completion and functionality are supported by the Ministry of Universities and Research (MUR) Department of Excellence funding, received by the Department of Translational Medicine. The budget was spent on sample processing and quality control equipment, the implementation of the storage capacity, and the commercial LIMS. A grant from "Fondo per l'edilizia universitaria e per le grandi attrezzature scien-

tifiche –2020” (MIUR) supported the acquisition of two MAPI2 semi-automated samples aliquoting systems, whereas the increase in storage capacity with the acquisition of an additional nitrogen tank was possible thanks to INFRA-P funding managed by Regione Piemonte (Italy).

The technical management of a biobank’s infrastructure and resources—such as storage facilities and sample/data processing—is central to maintaining quality, and it determines the relevance and success of a biobank, but requires trained personnel. However, the recruitment of dedicated and qualified personnel is a well-known challenge for biobanks. With the aim of guaranteeing a prospect of growth and stability for its research infrastructures, the UPO administration has established a stabilization program for technical staff. In particular, UPO Biobank has been equipped with dedicated trained personnel, represented by a Technical Manager (TM), a Data Manager (DM), a Quality Manager (QM), and a Study Nurse (SN). These figures have clear and defined duties that are critical to the proper functioning, maintenance, and growth of the biobank.

Another important challenge has been ensuring the continuity of UPO Biobank’s activity and monitoring 7/24. Toward this aim, UPO Biobank, in close collaboration with the UPO Innovation, Digitalization, and Process Quality division staff, is defining a Business Continuity Plan, which also includes technical personnel who can intervene when an emergency occurs. It is inevitable that the satisfaction of these needs strongly depends on the resource and personnel management policies of the institution in charge of the biobank. The disaster recovery plan is another delicate issue for Italian biobanks, frequently represented by biorepositories located in small and hard-to-reach buildings. UPO Biobank faced this problem by stipulating a contract with an external, private company located in the north of Italy that ensures to take action within 2 h in the event of malfunctioning of any cryogenic container, taking charge of the biological samples as long as the malfunctioning has been fixed, and maintaining the cold chain and traceability of the material.

A paramount aspect of building an institutional biobank is to focus attention not only on short-term biobank and stakeholders’ objectives, but also on medium- and long-term ones. Indeed, the future implementations of a biobank in terms of physical space, infrastructures, instrumentation, data storage, etc., should be preventively conceived to reduce the future costs and time spent. UPO Biobank, supporting population studies, has forecast a progressive increase in biological materials and associated data contribution to biobanks and has been designed to take the possibility of doubling its initial storage capacity into consideration.

Privacy and data security are pillars of a biobank’s organization, as discussed above. This implies durable cooperation with the institutional figures deputed to privacy and data protection. UPO Biobank foresaw a privacy-by-design approach and immediately worked on an ENISA, a DPIA, and a data protection policy in cooperation with the UPO DPO and Information and Communication Technologies division. Biobanks, to truly support quality research that serves the whole community, need to collect not only biological samples but also associated quality data. Therefore, the need to engage in a dialogue with local health authorities regarding the shared use, within the framework of the GDPR, of health and research data is becoming increasingly pressing. Several Italian biobanks, including UPO Biobank, are moving in this direction with a view to collaborating with regional and national institutions for the pursuit of actions aimed at improving public health and the acquisition of new knowledge that can be placed at the service of the whole community.

4. Discussion

Biobanks are powerful tools for biomedical research that represent the core of the scientific process, granting undeniable quality, impartiality, and ethics to scientific results. Academic biobanks, in particular, are integrated into an ideal environment aimed at promoting and sustaining multidisciplinary research that complements social and scientific finalities. By sustaining population studies, such as the Novara Cohort Study, UPO Biobank

will lay the foundations for a platform in support of epidemiological and social studies aimed at promoting healthy aging and public engagement in scientific research.

Moreover, UPO Biobank, as an institutional infrastructure, is sustaining the development of a collaborative network between the university and local healthcare services and authorities, including hospitals and local health units. The presence of this collaborative network will allow the implementation and consolidation of a scientific collaboration aimed at promoting health in the territory that can have a tangible impact through the implementation of innovative primary, secondary, and tertiary prevention measures. However, this goal could only be achieved with a constant exchange with the local healthcare services and authorities, together with a shared purpose of integrating biobanking into the clinical routine. UPO Biobank successfully cooperates with different clinical units of the Ospedale Maggiore della Carità in Novara and is working on a communication channel with the local healthcare authority with the purpose of expanding the efficient cooperation at the local and regional levels.

Finally, the current project's objective, the Excellence Project funded by the Italian Ministry of University and Research (MUR), to implement an information technology (IT) and artificial intelligence infrastructure to support UPO Biobank and NCS, will allow, in the future, a deep and integrated analysis of biobanked data.

5. Conclusions

Thanks to the standardized manipulation and preservation practices linked to organized data management, UPO Biobank is emerging as a dynamic and fast-growing facility in UPO with the potential to sustain local biomedical and translational research. As a member of the BBMRI.it network, the development and improvements to UPO Biobank are indeed outward-looking to expand collaboration and encourage the efficient and simple sharing of biological samples and data within the scientific community, in compliance with national and international laws and ethical guides.

Moreover, the UPO biobank is a technological tool for scientific innovation with a potentially strong social and economic impact on the territory. An infrastructure of this value may indeed contribute to R&D with local and international partners and attract further public and private investments in the Novara area.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/jpm13060911/s1>, Table S1: Biological material stored in UPO Biobank; Table S2: Associated data stored in UPO Biobank; Table S3: Age distribution of subjects.

Author Contributions: Conceptualization, V.B., E.M. and D.C.; formal analysis, V.B., E.M. and D.F.; data curation, V.B., E.M. and D.F.; writing—original draft preparation, V.B. and D.C.; writing—review and editing, V.B., E.M., C.A., S.C., C.R., A.A., M.V., J.V., D.F., A.R. and D.C.; supervision, D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian Ministry of Education, University and Research (MIUR) program “Departments of Excellence 2018–2022” AGING Project—Department of Translational Medicine, Università del Piemonte Orientale, by the Italian Ministry of University and Research (MUR) program “Departments of Excellence 2023–2027”, AGING Project—Department of Translational Medicine, Università del Piemonte Orientale, by POR FESR 2014–2020—Azione I.1a.5.1 Bando INFRA-P Sostegno a progetti per la realizzazione, il rafforzamento e l’ampliamento di IR pubbliche’ received by CAAD (cod. 321-13), and by “Fondo per l’edilizia universitaria e per le grandi attrezzature scientifiche per il periodo 2019–2033 (D.M. n. 1121 del 05/12/2019)”. The funders had no role in the study’s design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: The code of ethics, regulation, and informed consent of UPO Biobank have been approved by the Ethics Committee of the Ospedale Maggiore della Carità in Novara and by the DPO of the University. The studies supported by UPO Biobank have been approved by the competent Ethics Committees.

Informed Consent Statement: The prerequisite for the enrolment in these studies and the aims and purposes of UPO Biobank were depicted in the written informed consent. All the participants were asked to sign the written informed consent for the inclusion of their data and samples in UPO Biobank.

Data Availability Statement: The biological material and associated data stored in UPO Biobank can be obtained following a successful application and evaluation process. If the researcher's request is accepted by the TSC, a data and material transfer agreement is signed and both biological material and data can be supplied. Further details can be found at <https://biobank.uniupo.it/> (accessed on 24 November 2022).

Acknowledgments: The authors would like to thank the participants for entrusting UPO Biobank with their biological samples and associated data.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Ashley, E.A. Towards precision medicine. *Nat. Rev. Genet.* **2016**, *17*, 507–522. [CrossRef]
- Beckmann, J.S.; Lew, D. Reconciling evidence-based medicine and precision medicine in the era of big data: Challenges and opportunities. *Genome Med.* **2016**, *8*, 134. [CrossRef]
- Carrasco-Ramiro, F.; Peiró-Pastor, R.; Aguado, B. Human genomics projects and precision medicine. *Gene Ther.* **2017**, *24*, 551–561. [CrossRef]
- Abul-Husn, N.S.; Kenny, E.E. Personalized Medicine and the Power of Electronic Health Records. *Cell* **2019**, *177*, 58–69. [CrossRef]
- Kraus, V.B. Biomarkers as drug development tools: Discovery, validation, qualification and use. *Nat. Rev. Rheumatol.* **2018**, *14*, 354–362. [CrossRef]
- Hulsén, T.; Jamuar, S.S.; Moody, A.R.; Karnes, J.H.; Varga, O.; Hedensted, S.; Spreafico, R.; Hafler, D.A.; McKinney, E.F. From Big Data to Precision Medicine. *Front. Med.* **2019**, *6*, 34. [CrossRef]
- Barnes, R.O.; Watson, P. Precision medicine: Driving the evolution of biobanking quality. *Heal. Manag. Forum* **2020**, *33*, 102–106. [CrossRef]
- Loft, S.; Poulsen, H.E. Cancer risk and oxidative DNA damage in man. *J. Mol. Med.* **1996**, *74*, 297–312. [CrossRef]
- Liu, A.; Pollard, K. *Biobanking for Personalized Medicine*; Springer: Cham, Switzerland, 2015; Volume 864, pp. 55–68. [CrossRef]
- Linsen, L.; Vanhees, K.; Vanoppen, E.; Ulenaers, K.; Driessens, S.; Penders, J.; Somers, V.; Stinissen, P.; Rummens, J.-L. Raising to the Challenge: Building a Federated Biobank to Accelerate Translational Research—The University Biobank Limburg. *Front. Med.* **2019**, *6*, 224. [CrossRef]
- Mendy, M.; Caboux, E.; Wild, C.P.; Herrero, R.; Accardi-Gheit, R.; Clifford, G.; Françon, E.; Kesminiene, A.; McKay, J.; Rinaldi, S.; et al. Centralization of the IARC Biobank: Combining Multiple Sample Collections into a Common Platform. *Biopreservation Biobanking* **2019**, *17*, 433–443. [CrossRef]
- Amin, L.; Olesen, A.; Mahadi, Z.; Ibrahim, M. Current Status and Future Challenges of Biobank Research in Malaysia. *Asian Bioeth. Rev.* **2021**, *13*, 297–315. [CrossRef] [PubMed]
- Bürtin, F.; Matschos, S.; Prall, F.; Mullins, C.S.; Krohn, M.; Linnebacher, M. Creation and Maintenance of a Living Biobank—How We Do It. *J. Vis. Exp.* **2021**, e62065. [CrossRef]
- Kanakoglou, D.S.; Pampalou, A.; Vrachnos, D.M.; Karatrasoglou, E.A.; Zouki, D.N.; Dimonitsas, E.; Klonou, A.; Kokla, G.; Theologi, V.; Christofidou, E.; et al. Laying the groundwork for the Biobank of Rare Malignant Neoplasms at the service of the Hellenic Network of Precision Medicine on Cancer. *Int. J. Oncol.* **2022**, *60*, 31. [CrossRef] [PubMed]
- Wiskott, K.; Gilardi, F.; Michaud, K.; Augsburg, M.; Castiglioni, C.; Carminati, A.; Grabherr, S.; Thomas, A.; Fracasso, T. Creation of a Forensic Pathology Biobank in Switzerland: Which issues and research opportunities? *Int. J. Leg. Med.* **2022**, *136*, 919–922. [CrossRef] [PubMed]
- Huppertz, B.; Bayer, M.; Macheiner, T.; Sargsyan, K. Biobank Graz: The Hub for Innovative Biomedical Research. *Open J. Bioresour.* **2016**, *3*, 366. [CrossRef]
- Parodi, B. Biobanks: A Definition, Ethics, Law and Governance of Biobanking. *Int. Libr. Ethics Law Technol.* **2015**, *14*, 15–19. [CrossRef]
- Paskal, W.; Paskal, A.M.; Debski, T.; Gryziak, M.; Jaworowski, J. Aspects of Modern Biobank Activity – Comprehensive Review. *Pathol. Oncol. Res.* **2018**, *24*, 771–785. [CrossRef]
- Malsagova, K.; Kopylov, A.; Stepanov, A.; Butkova, T.; Sinitsyna, A.; Izotov, A.; Kaysheva, A. Biobanks—A Platform for Scientific and Biomedical Research. *Diagnostics* **2020**, *10*, 485. [CrossRef]
- Müller, H.; Dagher, G.; Loibner, M.; Stumptner, C.; Kungl, P.; Zatloukal, K. Biobanks for life sciences and personalized medicine: Importance of standardization, biosafety, biosecurity, and data management. *Curr. Opin. Biotechnol.* **2020**, *65*, 45–51. [CrossRef]
- Pokrovskaya, M.S.; Sivakova, O.V.; Efimova, I.A.; Meshkov, A.N.; Metelskaya, V.A.; Shalnova, S.A.; Drapkina, O.M.; Sergeevna, P.M.; Victorovna, S.O.; Aleksandrovna, E.I.; et al. Biobanking as a necessary tool for research in the field of personalized medicine in the scientific medical center. *Pers. Med.* **2019**, *16*, 501–509. [CrossRef]

22. Coppola, L.; Cianflone, A.; Grimaldi, A.M.; Incoronato, M.; Bevilacqua, P.; Messina, F.; Baselice, S.; Soricelli, A.; Mirabelli, P.; Salvatore, M. Biobanking in health care: Evolution and future directions. *J. Transl. Med.* **2019**, *17*, 1–18. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Rønningen, K.S.; Paltiel, L.; Meltzer, H.M.; Nordhagen, R.; Lie, K.K.; Hovengen, R.; Haugen, M.; Nystad, W.; Magnus, P.; Hoppin, J.A. The biobank of the Norwegian mother and child cohort Study: A resource for the next 100 years. *Eur. J. Epidemiol.* **2006**, *21*, 619–625. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Pattaro, C.; Gögele, M.; Mascalconi, D.; Melotti, R.; Schwienbacher, C.; De Grandi, A.; Foco, L.; D’elia, Y.; Linder, B.; Fuchsberger, C.; et al. The Cooperative Health Research in South Tyrol (CHRIS) study: Rationale, objectives, and preliminary results. *J. Transl. Med.* **2015**, *13*, 348. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Bonaccio, M.; Costanzo, S.; Di Castelnuovo, A.; Gialluisi, A.; Ruggiero, E.; De Curtis, A.; Persichillo, M.; Cerletti, C.; Donati, M.B.; de Gaetano, G.; et al. Increased Adherence to a Mediterranean Diet Is Associated With Reduced Low-Grade Inflammation after a 12.7-Year Period: Results From the Moli-sani Study. *J. Acad. Nutr. Diet.* **2022**, *123*, 783–795.e7. [\[CrossRef\]](#)
26. Rusk, N. The UK Biobank. *Nat. Methods* **2018**, *15*, 1001. [\[CrossRef\]](#)
27. Al Thani, A.; Fthenou, E.; Paparrodopoulos, S.; Al Marri, A.; Shi, Z.; Qafoud, F.; Afifi, N. Qatar Biobank Cohort Study: Study Design and First Results. *Am. J. Epidemiol.* **2019**, *188*, 1420–1433. [\[CrossRef\]](#)
28. Haudum, C.W.; Kolesnik, E.; Colantonio, C.; Mursic, I.; Url-Michitsch, M.; Tomaschitz, A.; Glantschnig, T.; Hutz, B.; Lind, A.; Schweighofer, N.; et al. Cohort profile: ‘Biomarkers of Personalised Medicine’ (BioPersMed): A single-centre prospective observational cohort study in Graz/Austria to evaluate novel biomarkers in cardiovascular and metabolic diseases. *BMJ Open* **2022**, *12*, e058890. [\[CrossRef\]](#)
29. Baronti, C.; Lieutaud, P.; Bardsley, M.; de Lamballerie, X.; Resman Rus, K.; Korva, M.; Petrovec, M.; Avsic-Zupanc, T.; Matusali, G.; Meschi, S.; et al. The Importance of Biobanking for Response to Pandemics Caused by Emerging Viruses: The European Virus Archive As an Observatory of the Global Response to the Zika Virus and COVID-19 Crisis. *Biopreservation Biobanking* **2020**, *18*, 561–569. [\[CrossRef\]](#)
30. Peeling, R.W.; Boeras, D.; Wilder-Smith, A.; Sall, A.; Nkengasong, J. Need for sustainable biobanking networks for COVID-19 and other diseases of epidemic potential. *Lancet Infect. Dis.* **2020**, *20*, e268–e273. [\[CrossRef\]](#)
31. Carress, H.; Lawson, D.J.; Elhaik, E. Population genetic considerations for using biobanks as international resources in the pandemic era and beyond. *BMC Genom.* **2021**, *22*, 351. [\[CrossRef\]](#)
32. Bezzini, D.; Schiavetti, I.; Manacorda, T.; Franzone, G.; Battaglia, M.A. First Wave of COVID-19 Pandemic in Italy: Data and Evidence. *Adv. Exp. Med. Biol.* **2021**, *1353*, 91–113. [\[CrossRef\]](#) [\[PubMed\]](#)
33. WHO Coronavirus (COVID-19) Dashboard. Available online: <https://covid19.who.int/> (accessed on 26 January 2023).
34. Borgogna, C.; Bruna, R.; Griffante, G.; Martuscelli, L.; De Andrea, M.; Ferrante, D.; Patriarca, A.; Mahmoud, A.M.; Ucciero, M.A.M.; Gaidano, V.; et al. Induction of robust humoral immunity against SARS-CoV-2 after vaccine administration in previously infected haematological cancer patients. *Br. J. Haematol.* **2022**, *199*, 463–467. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Borgogna, C.; Bruna, R.; Griffante, G.; Martuscelli, L.; De Andrea, M.; Ferrante, D.; Patriarca, A.; Mahmoud, A.M.; Gaidano, V.; Marchetti, M.; et al. Patterns of neutralizing humoral response to SARS-CoV-2 infection among hematologic malignancy patients reveal a robust immune response in anti-cancer therapy-naïve patients. *Blood Cancer J.* **2022**, *12*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Griffante, G.; Chandel, S.; Ferrante, D.; Caneparo, V.; Capello, D.; Bettio, V.; Borgogna, C.; Aleni, C.; Esposito, S.; Sarro, A.; et al. Persistence of Neutralizing Antibodies to SARS-CoV-2 in First Wave Infected Individuals at Ten Months Post-Infection: The UnIRSA Cohort Study. *Viruses* **2021**, *13*, 2270. [\[CrossRef\]](#)
37. Campani, D.; De Luca, E.; Bassi, E.; Busca, E.; Airoidi, C.; Barisone, M.; Canonico, M.; Contaldi, E.; Capello, D.; De Marchi, F.; et al. The prevention of falls in patients with Parkinson’s disease with in-home monitoring using a wearable system: A pilot study protocol. *Aging Clin. Exp. Res.* **2022**, *34*, 3017–3024. [\[CrossRef\]](#)
38. Bettio, V.; Mazzucco, E.; Antona, A.; Cracas, S.; Varalda, M.; Venetucci, J.; Bruno, S.; Chiabotto, G.; Venegoni, C.; Vasile, A.; et al. Extracellular vesicles from human plasma for biomarkers discovery: Impact of anticoagulants and isolation techniques. *PLoS ONE* **2023**, *18*, e0285440. [\[CrossRef\]](#)
39. Aleni, C.; Rinaldi, C.; Bettio, V.; Mazzucco, E.; Antona, A.; Meini, C.; Loria, E.; Bonvicini, P.; Cracas, S.V.; Caristia, S.; et al. Public Attitude towards Biobanking: An Italian University Survey. *Int. J. Environ. Res. Public Health* **2022**, *19*, 13041. [\[CrossRef\]](#)
40. Bellan, M.; Apostolo, D.; Albè, A.; Crevola, M.; Errica, N.; Ratano, G.; Tonello, S.; Minisini, R.; D’onghia, D.; Baricich, A.; et al. Determinants of long COVID among adults hospitalized for SARS-CoV-2 infection: A prospective cohort study. *Front. Immunol.* **2022**, *13*, 1038227. [\[CrossRef\]](#)
41. Zhang, P.-F.; Zheng, X.-H.; Li, X.-Z.; Sun, L.; Jia, W.-H. Informatics Management of Tumor Specimens in the Era of Big Data: Challenges and Solutions. *Biopreservation Biobanking* **2021**, *19*, 531–542. [\[CrossRef\]](#)
42. Elliott, P.; Peakman, T.C.; Biobank, O.B.O.U. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. *Leuk. Res.* **2008**, *37*, 234–244. [\[CrossRef\]](#)
43. Baust, J.G.; Snyder, K.K.; Van Buskirk, R. Integrating Molecular Control to Improve Cryopreservation Outcome. *Biopreservation Biobanking* **2017**, *15*, 134–141. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Viganò, M.; Budelli, S.; Lavazza, C.; Montemurro, T.; Montelatici, E.; De Cesare, S.; Lazzari, L.; Orlandi, A.R.; Lunghi, G.; Giordano, R. Tips and Tricks for Validation of Quality Control Analytical Methods in Good Manufacturing Practice Mesenchymal Stromal Cell Production. *Stem Cells Int.* **2018**, *2018*, 3038565. [\[CrossRef\]](#) [\[PubMed\]](#)

45. Li, R.; Johnson, R.; Yu, G.; McKenna, D.H.; Hubel, A. Preservation of cell-based immunotherapies for clinical trials. *Cytotherapy* **2019**, *21*, 943–957. [CrossRef] [PubMed]
46. Available online: <https://www.bbmri-eric.eu/> (accessed on 8 November 2022).
47. Mayrhofer, M.T.; Holub, P.; Wutte, A.; Litton, J.-E. BBMRI-ERIC: The novel gateway to biobanks. *Bundesgesundheitsblatt-Gesundh.-Gesundh.* **2016**, *59*, 379–384. [CrossRef]
48. Litton, J.-E. Launch of an Infrastructure for Health Research: BBMRI-ERIC. *Biopreservation Biobanking* **2018**, *16*, 233–241. [CrossRef] [PubMed]
49. Linsen, L.; T'Joene, V.; Van Der Straeten, C.; Van Landuyt, K.; Marbaix, E.; Bekaert, S.; Ectors, N. Biobank Quality Management in the BBMRI.be Network. *Front. Med.* **2019**, *6*, 141. [CrossRef]
50. Moutham, L.; Garrison, S.J.; Archer, D.L.; Castelhamo, M.G. A Biobank's Journey: Implementation of a Quality Management System and Accreditation to ISO 20387. *Biopreservation Biobanking* **2021**, *19*, 163–170. [CrossRef]
51. Dagher, G. Quality matters: International standards for biobanking. *Cell Prolif.* **2022**, *55*, e13282. [CrossRef] [PubMed]
52. Permenter, J.; Ishwar, A.; Rounsavall, A.; Smith, M.; Faske, J.; Sailey, C.J.; Alfaro, M.P. Quantitative analysis of genomic DNA degradation in whole blood under various storage conditions for molecular diagnostic testing. *Mol. Cell. Probes* **2015**, *29*, 449–453. [CrossRef]
53. Burla, B.; Arita, M.; Arita, M.; Bendt, A.K.; Cazenave-Gassiot, A.; Dennis, E.A.; Ekroos, K.; Han, X.; Ikeda, K.; Liebisch, G.; et al. MS-based lipidomics of human blood plasma: A community-initiated position paper to develop accepted guidelines. *J. Lipid Res.* **2018**, *59*, 2001–2017. [CrossRef]
54. Lan, J.; Galindo, A.N.; Doecke, J.; Fowler, C.; Martins, R.N.; Rainey-Smith, S.R.; Cominetti, O.; Dayon, L. Systematic Evaluation of the Use of Human Plasma and Serum for Mass-Spectrometry-Based Shotgun Proteomics. *J. Proteome Res.* **2018**, *17*, 1426–1435. [CrossRef] [PubMed]
55. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* **2018**, *7*, 1535750. [CrossRef] [PubMed]
56. Mendy, M.; Caboux, E.; Lawlor, R.T.; Wright, J.; Wild, C.P. *Common Minimum Technical Standards and Protocols for Biobanks Dedicated to Cancer Research*; International Agency for Research on Cancer, World Health Organization: Lyon, France, 2017; ISBN 9789283224631.
57. Guo, J.; Liu, D.; Zhang, X.; Johnson, H.; Feng, X.; Zhang, H.; Wu, A.H.B.; Chen, L.; Fang, J.; Xiao, Z.; et al. Establishing a Urine-Based Biomarker Assay for Prostate Cancer Risk Stratification. *Front. Cell Dev. Biol.* **2020**, *8*, 597961. [CrossRef]
58. Costa, M.M.; Benoit, N.; Dormoi, J.; Amalvict, R.; Gomez, N.; Tissot-Dupont, H.; Million, M.; Pradines, B.; Granjeaud, S.; Almeras, L. Salivette, a relevant saliva sampling device for SARS-CoV-2 detection. *J. Oral Microbiol.* **2021**, *13*, 1920226. [CrossRef] [PubMed]
59. Castro, T.; Sabalza, M.; Barber, C.; Abrams, W.; Da Costa, A.C.; Milagres, F.A.D.P.; Braz-Silva, P.H.; Malamud, D.; Gallottini, M. Rapid diagnosis of Zika virus through saliva and urine by Loop-mediated isothermal amplification (LAMP). *J. Oral Microbiol.* **2018**, *10*, 1510712. [CrossRef] [PubMed]
60. Isho, B.; Abe, K.T.; Zuo, M.; Jamal, A.J.; Rathod, B.; Wang, J.H.; Li, Z.; Chao, G.; Rojas, O.L.; Bang, Y.M.; et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci. Immunol.* **2020**, *5*, eabe5511. [CrossRef] [PubMed]
61. Rush, A.; Catchpole, D.R.; Ling, R.; Searles, A.; Watson, P.H.; Byrne, J.A. Improving Academic Biobank Value and Sustainability Through an Outputs Focus. *Value Health* **2020**, *23*, 1072–1078. [CrossRef]
62. Zohouri, M.; Ghaderi, A. The Significance of Biobanking in the Sustainability of Biomedical Research: A Review. *Iran. Biomed. J.* **2020**, *24*, 206–213. [CrossRef]
63. Myskja, B.K. Lay expertise: Why involve the public in biobank governance? *Genom. Soc. Policy* **2007**, *3*, 1. [CrossRef]
64. Lemke, A.A.; Harris-Wai, J.N. Stakeholder engagement in policy development: Challenges and opportunities for human genomics. *Anesth. Analg.* **2015**, *17*, 949–957. [CrossRef]
65. Brizi, M.R. Biobank Research and Data Protection Issues Under the GDPR. *Pers. Med. Mak.* **2022**, *3*, 269–282. [CrossRef]
66. Available online: <https://biobank.uniupo.it/> (accessed on 24 November 2022).
67. Klingler, C.; von Jagwitz-Biegnitz, M.; Baber, R.; Becker, K.-F.; Dahl, E.; Eibner, C.; Fuchs, J.; Groenewold, M.K.; Hartung, M.L.; Hummel, M.; et al. Stakeholder engagement to ensure the sustainability of biobanks: A survey of potential users of biobank services. *Eur. J. Hum. Genet.* **2021**, *30*, 1344–1354. [CrossRef] [PubMed]
68. Scudellari, M. Biobank managers bemoan underuse of collected samples. *Nat. Med.* **2013**, *19*, 253. [CrossRef] [PubMed]
69. Peloquin, D.; DiMaio, M.; Bierer, B.; Barnes, M. Disruptive and avoidable: GDPR challenges to secondary research uses of data. *Eur. J. Hum. Genet.* **2020**, *28*, 697–705. [CrossRef]
70. Krekora-Zaja, D.; Marciniak, B.; Pawlikowski, J. Recommendations for Creating Codes of Conduct for Processing Personal Data in Biobanking Based on the GDPR art.40. *Front. Genet.* **2021**, *12*. [CrossRef] [PubMed]
71. Shabani, M.; Chassang, G.; Marelli, L. The Impact of the GDPR on the Governance of Biobank Research. *GDPR Biobanking Law Gov. Technol. Ser.* **2021**, *43*, 45–60. [CrossRef]

72. Article 29 Data Protection Working Party Article 29 Working Party Guidelines on Data Protection Impact Assessment (DPIA); Eu-ropean Commission: Brussels, Belgium, WP248 Rev.01. Available online: <https://policycommons.net/artifacts/2033929/article-29-data-protection-working-party-subject/2786372/> (accessed on 25 February 2022).
73. Article 29 Data Protection Working Party Guidelines on Data Protection Impact Assessment (DPIA) and Determining Whether Processing Is “Likely to Result in a High Risk” for the Purposes of Regulation 2016/679 (WP 248 Rev.01). Adopted on 4 April 2017. As Last Revised and Adopted on 4 October 2017. Available online: <https://ec.europa.eu/newsroom/article29/items/611236> (accessed on 25 February 2022).
74. BBMRI-ERIC BBMRI-ERIC Joint Comments to the Article 29 Working Party Guidelines on Consent under Regulation 2016/679 (Wp259) and Transparency under Regulation 2016/679 (Wp260). National Node-Italy, p. 24. Available online: https://www.bbmri-eric.eu/wp-content/uploads/WP29_consent-joint-comments_BBMRI-ERIC_as-submitted.pdf (accessed on 25 February 2022).
75. Staunton, C.; Slokenberga, S.; Parziale, A.; Mascalconi, D. Appropriate Safeguards and Article 89 of the GDPR: Considerations for Biobank, Databank and Genetic Research. *Front. Genet.* **2022**, *13*, 719317. [CrossRef]
76. OECD Ensuring Data Privacy as We Battle COVID-19, OECD Policy Responses to Coronavirus (COVID-19); Paris, France. 2020. Available online: <https://www.oecd.org/coronavirus/policy-responses/ensuring-data-privacy-as-we-battle-covid-19-36c2f31e/> (accessed on 25 February 2022).
77. Williams, Y. Bruges Regional Integration & Global Governance Papers Shifting Between Hegemony and Dominance? A Neo-Gramscian Analysis of the EU as a Structural Foreign Policy Actor: The Singular Case of the Cariforum-EU Economic Partnership Agreement. 2014. Available online: https://www.academia.edu/download/35507531/brigg_1_2014_williams.pdf (accessed on 25 February 2022).
78. Autorizzazione n. 8/2016-Autorizzazione Generale al Trattamento Dei Dati Genetici-15 Dicembre 2016. Available online: <https://www.garanteprivacy.it/home/docweb/-/docweb-display/docweb/5803688> (accessed on 25 February 2022).
79. Autorizzazione n. 9/2016-Autorizzazione Generale al Trattamento Dei Dati Personali Effettuato per Scopi Di Ricerca Scientifica-15 Dicembre 2016. Available online: <https://www.garanteprivacy.it/home/docweb/-/docweb-display/docweb/5805552> (accessed on 25 February 2022).
80. Smart, A.; Tutton, R.; Martin, P.; Ellison, G.T.; Ashcroft, R. The Standardization of Race and Ethnicity in Biomedical Science Editorials and UK Biobanks. *Soc. Stud. Sci.* **2008**, *38*, 407–423. [CrossRef]
81. Tutton, R. Banking expectations: The promises and problems of biobanks. *Pers. Med.* **2007**, *4*, 463–469. [CrossRef]
82. Bryzgalina, E.V.; Alasania, K.Y.; Varkhotov, T.A.; Gavrilenko, S.M.; Shkomova, E.M. The social dimension of biobanking: Objectives and challenges. *Life Sci. Soc. Policy* **2017**, *13*, 15. [CrossRef]
83. Tutton, R. Governing UK Biobank: The importance of ensuring public trust. *Trends Biotechnol.* **2004**, *22*, 284–285. [CrossRef]
84. Milne, R.; Sorbie, A.; Dixon-Woods, M. What can data trusts for health research learn from participatory governance in biobanks? *J. Med. Ethics* **2021**, *48*, 323–328. [CrossRef] [PubMed]
85. Jones, K.H.; Laurie, G.; Stevens, L.; Dobbs, C.; Ford, D.V.; Lea, N. The other side of the coin: Harm due to the non-use of health-related data. *Int. J. Med. Inform.* **2017**, *97*, 43–51. [CrossRef] [PubMed]
86. Steinsbekk, K.S.; Myskja, B.K.; Solberg, B. Broad consent versus dynamic consent in biobank research: Is passive participation an ethical problem? *Eur. J. Hum. Genet.* **2013**, *21*, 897–902. [CrossRef] [PubMed]
87. Mikkelsen, R.B.; Gjerris, M.; Waldemar, G.; Sandøe, P. Broad consent for biobanks is best—provided it is also deep. *BMC Med. Ethics* **2019**, *20*, 1–12. [CrossRef]
88. Strech, D.; Bein, S.; Brumhard, M.; Eisenmenger, W.; Glinicke, C.; Herbst, T.; Jahns, R.; von Kielmansegg, S.; Schmidt, G.; Taupitz, J.; et al. A template for broad consent in biobank research. Results and explanation of an evidence and consensus-based development process. *Eur. J. Med. Genet.* **2016**, *59*, 295–309. [CrossRef]
89. Bosio, F.; Barazzetti, G.; Koutaissoff, D.; Spencer, B. Patients’ decision to contribute to a biobank in the light of the patient-recruiter relationship—a qualitative study of broad consent in a hospital setting. *J. Community Genet.* **2020**, *12*, 15–25. [CrossRef] [PubMed]
90. Cargill, S.S. Biobanking and the Abandonment of Informed Consent: An Ethical Imperative. *Public Health Ethics* **2016**, *9*, 255–263. [CrossRef]
91. Caulfield, T.; Murdoch, B. Genes, cells, and biobanks: Yes, there’s still a consent problem. *PLoS Biol.* **2017**, *15*, e2002654. [CrossRef]
92. Eisenhauer, E.R.; Tait, A.R.; Rieh, S.Y.; Arslanian-Engoren, C. Participants’ Understanding of Informed Consent for Biobanking: A Systematic Review. *Clin. Nurs. Res.* **2017**, *28*, 30–51. [CrossRef]
93. Manson, N.C. The ethics of biobanking: Assessing the *right to control* problem for broad consent. *Bioethics* **2019**, *33*, 540–549. [CrossRef]
94. Hallinan, D. Broad consent under the GDPR: An optimistic perspective on a bright future. *Life Sci. Soc. Policy* **2020**, *16*, 1. [CrossRef] [PubMed]
95. Gottweis, H.; Gaskell, G.; Starkbaum, J. Connecting the public with biobank research: Reciprocity matters. *Nat. Rev. Genet.* **2011**, *12*, 738–739. [CrossRef] [PubMed]
96. Ronaldson, A.; de la Torre, J.A.; Gaughran, F.; Bakolis, I.; Hatch, S.L.; Hotopf, M.; Dregan, A. Prospective associations between vitamin D and depression in middle-aged adults: Findings from the UK Biobank cohort. *Psychol. Med.* **2020**, *52*, 1866–1874. [CrossRef] [PubMed]

97. Spector-Bagdady, K.; De Vries, R.G.; Gornick, M.G.; Shuman, A.G.; Kardia, S.; Platt, J. Encouraging Participation And Transparency In Biobank Research. *Health Aff.* **2018**, *37*, 1313–1320. [[CrossRef](#)] [[PubMed](#)]
98. Dive, L.; Critchley, C.; Otlowski, M.; Mason, P.; Wiersma, M.; Light, E.; Stewart, C.; Kerridge, I.; Lipworth, W. Public trust and global biobank networks. *BMC Med. Ethic* **2020**, *21*, 1–9. [[CrossRef](#)] [[PubMed](#)]
99. Ursin, L.; Ytterhus, B.; Christensen, E.; Skolbekken, J.-A. «If you give them your little finger, they'll tear off your entire arm»: Losing trust in biobank research. *Med. Health Care Philos.* **2020**, *23*, 565–576. [[CrossRef](#)]
100. Brezzi, M.; González, S.; Nguyen, D.; Prats, M. *An Updated OECD Framework on Drivers of Trust in Public Institutions to Meet Current and Future Challenges*; OECD: Paris, France, 2021. [[CrossRef](#)]
101. Chalmers, D.; Nicol, D.; Kaye, J.; Bell, J.; Campbell, A.V.; Ho, C.W.L.; Kato, K.; Minari, J.; Ho, C.-H.; Mitchell, C.; et al. Has the biobank bubble burst? Withstanding the challenges for sustainable biobanking in the digital era. *BMC Med. Ethic* **2016**, *17*, 1–14. [[CrossRef](#)]
102. Fernández, I.C.; Merino, I.G.; Muñoz-Fernández, M. Assessing and measuring financial sustainability model of the Spanish HIV HGM BioBank. *J. Transl. Med.* **2020**, *18*, 6. [[CrossRef](#)]
103. Odeh, H.; Miranda, L.; Rao, A.; Vaught, J.; Greenman, H.; McLean, J.; Reed, D.; Memon, S.; Fombonne, B.; Guan, P.; et al. The Biobank Economic Modeling Tool (BEMT): Online Financial Planning to Facilitate Biobank Sustainability. *Biopreservation Biobanking* **2015**, *13*, 421–429. [[CrossRef](#)]
104. Matzke, L.; Dee, S.; Bartlett, J.; Damaraju, S.; Graham, K.; Johnston, R.; Mes-Masson, A.-M.; Murphy, L.; Shepherd, L.; Schacter, B.; et al. A Practical Tool for Modeling Biospecimen User Fees. *Biopreservation Biobanking* **2014**, *12*, 234–239. [[CrossRef](#)] [[PubMed](#)]
105. Kinkorová, J.; Topolčan, O. Biobanks in Horizon 2020: Sustainability and attractive perspectives. *EPMA J.* **2018**, *9*, 345–353. [[CrossRef](#)] [[PubMed](#)]
106. Available online: <https://Horizoneurope.Apre.It/> (accessed on 25 February 2022).

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.



The Dedalo Project, a Community-based Prevention Program for the Promotion of Healthy Behaviors in Adult Population: Model Description and Target Population Assessment

Sara Bortoluzzi¹ · Alessandro Coppo² · Daniela Alessi² · Stefano Parovina³ · Sara Napoletano³ · Irene Ammazzagatti³, et al. [full author details at the end of the article]

Accepted: 7 June 2022 / Published online: 14 July 2022
© The Author(s) 2022

Abstract

Noncommunicable diseases (NCDs) are the leading global cause of death. The Italian National Prevention Plan (INPP) highlights the importance of health promotion and NCD prevention while avoiding health inequalities. In line with the INPP guidelines, we initiated a multicomponent community-based intervention program, named the Dedalo project, to promote healthy and active aging among population living around the Vercelli's municipality, Italy. A cross-sectional analysis, that used the baseline data of a longitudinal study of the project, evaluated the program's ability to enroll participants who represent the socioeconomic conditions present in the municipality. To this end, we compared the main social characteristics and behaviors of 40–74-year-old subjects ($n = 155$), who had attended at least one Dedalo activity, to those of same age individuals ($n = 124$) randomly extracted from the general population. We found that most participants were women (81.3%) and had a higher SES compared to the general population. Furthermore, they were healthier—OR 0.52, 95% CI 0.31–0.87 for self-reported diseases—and displayed healthier behaviors—OR 0.20, 95% CI 0.08–0.46 for smokers; 0.32 95%, CI 0.16–0.64 for fruit/vegetable consumers; and 0.36 95% CI 0.20–0.64 for sweet beverages consumers. Overall, our initial evaluation indicates that the Dedalo project has so far struggled to enroll individuals with low SES, men with any SES, and subjects displaying unhealthy behaviors, thereby failing to meet the INPP goal of preventing NCDs while avoiding health disparities. Thus, efforts should be made to ensure that this community-based intervention program can effectively reach all the target population, in particular those individuals most exposed to behavioral risk factors.

Keywords Healthy aging · Socioeconomic differences · Noncommunicable diseases · Community · Prevention · Health inequalities

Introduction

Noncommunicable diseases (NCDs) are defined by the World Health Organization (WHO) as "health problems that require continuous treatment over a period of time from years to decades" due to a combination of genetic, physiological, environmental, and behavioral factors (Gakidou et al., 2017). Currently, NCDs are the leading global cause of death (Skolnik, 2018), causing more deaths than all other causes combined, with over 77% of all NCD deaths occurring in low- and middle-income countries (WHO, 2011).

Among the six WHO regions, Europe is the most affected by NCDs, and the impact of the major NCDs—i.e., diabetes, cardiovascular diseases, cancer, chronic respiratory diseases, and mental disorders—is increasing at an alarming rate, accounting for an estimated 86% of deaths and 77% of disease burden [evaluated in Disability-Adjusted Life Years—DALY (Benziger et al., 2016)] (Gakidou et al., 2017).

In the pre-COVID-19 era, most deaths in Italy were caused by NCDs, with ischemic heart diseases, stroke, and Alzheimer occupying the first three positions in the ranking of death causes (Vos et al., 2020). In 2019, despite decreasing trends, the majority of risk factors were found to be mainly related to unhealthy behaviors: tobacco use, alcohol intake, high fasting plasma glucose, high blood pressure, overweight, diet (Murray et al., 2020).

The aging of the Western population is typically accompanied by increased NCD burden (Vos et al., 2020). According to the estimates of the Italian National Institute of Statistics (Istat), in 2019, 32.3% of the Italian elderly suffered from chronic diseases, 6.9% had impaired autonomy in their daily life (care and domestic tasks), significant enough to require caregivers, and 4.2% of them had dementia or Alzheimer's disease (Istat, 2021). As the number of old age people will continue to increase for the foreseeable future, reaching an estimated 25% of the general population by 2027 (Istat, 2018), the prevalence of old age-associated NCDs and their ensuing financial burden is expected to grow exponentially (Istat, 2018).

In many high-income countries, such as Italy, NCDs are also a major cause of health disparities. The social determinants have in fact been shown to influence the distribution of the four main behavioral risk factors (unhealthy diet, physical inactivity, tobacco smoking, and excess alcohol consumption) (Marmot & Bell, 2019). Furthermore, individuals with high socioeconomic status (SES) are more likely to join prevention programs against NCDs (WHO, 2010), leading to a further increase in health disparities. In light of this, the Italian National Prevention Plan (INPP) advocates the design and implementation of evidence-based health promotion and preventive intervention programs capable of reducing health inequalities (Ministero della Salute—Direzione Generale della Prevenzione Sanitaria 2020).

In this scenario, the Italian PASSI surveillance system has revealed that, among individuals residing in the municipality of Vercelli, Italy (47,000 inhabitants), the 33% of sedentary people, the 45% with a BMI ≥ 25 (overweight/obese),

and the 24% who declared to smoke regularly tobacco were in the age class 50–69 years (Epicentro - ISS, 2019a). Thus, in order to counteract a likely rise in the prevalence of NCDs, in 2019, the Local Health Authority (LHA) of Vercelli, together with University of Piemonte Orientale and the municipality of Vercelli, launched the Dedalo project, a community-based intervention program aimed to promote healthy and active aging through the development of cognitive and relational abilities. It takes place in the community setting involving and coordinating local agencies and stakeholders that can contribute to offering prevention interventions to citizens (ASL VC, 2021). An evaluation study was also put in place in order to evaluate the impact on health of the project.

As INPP guidelines require that public health intervention for prevention and control of NCDs must ensure a concomitant reduction in health disparities, the aim of the study is to assess the Dedalo project's ability to enroll participants whose socioeconomic status reflects that of the general population. The implications of our findings are discussed in light of future program improvements.

Methods

Study Design

We cross-sectionally analyzed data of the baseline of a prospective study initiated in March 2018 to evaluate the effectiveness of the Dedalo project in promoting healthy habits and behaviors among Italians aged 40–74 years.

The protocol of the study is registered at ClinicalTrial.Gov (clinical trial registration No. NCT03939754). The ethical approval was obtained from the Ethics Committee of Alessandria “Comitato Etico Interaziendale ASO SS. Antonio e Biagio e C. Arrigo” (No. AsIVC.Med.18.02).

Population, Enrollment, and Inclusion Criteria

All participants in the Dedalo project from March to December 2018, aged 40–74 years, were deemed eligible for this study. The study participants were enrolled during a Dedalo activity only after obtaining their written consent. Exclusion criteria were language other than Italian and the refusal to sign the informed consent form before enrollment. The control group was a random sample of individuals aged 40–74 extracted from the population registry of the municipality of Vercelli, stratified by age classes and sex. These subjects were enrolled through computer-aided telephone interview (CATI). Subjects who had participated in any Dedalo activity were deemed ineligible for the control group.

The target number of people to be enrolled in the study was 428 (214 for each group). This sample size was estimated considering the following data from literature: i) a 28–43% increase in prevalence of physically active adult population (data of Emp-H study—<http://www.emp-h-project.eu/>); ii) a 33% prevalence of inactive adult people living in Vercelli (Epicentro - ISS, 2019a); iii) an expected reduction of

sedentary people by 35% after the intervention. The confidence intervals (CIs) and the power chosen were 95% and 80%, respectively. However, of the 345 participants in the Dedalo activities deemed eligible for the study, only 155 subjects completed the baseline interview. Thus, the total number of study participants was 369: 155 for the exposure group and 214 for the control group.

Intervention

The Dedalo project was implemented thanks to a joint effort of the Vercelli's LHA, the University of Piemonte Orientale (UPO), the municipality of Vercelli, and several other local organizations. It aims to promote healthy aging among the people residing in the municipality of Vercelli. The project takes place in the community setting developing community networks and mobilizing local stakeholders around a supply of "preventive opportunities" for the population (by networking building, coordination of actors and dissemination / communication of the Dedalo events to the population). To this end, the project has created a network of public and private entities, sports and cultural associations, volunteer associations, and other stakeholders to identify those activities that would best improve habits related to diet and physical activity in order to mitigate the risk of NCDs among the target population. The selected activities meeting the scientific standards of effectiveness are then offered to the community (Gottfredson et al., 2015).

Overall, the project is a multicomponent intervention characterized by social marketing and community coalition building organized in four paths: "Good diet, good health", "Let's work out together", "Surprise and amazement", and "Discovery of the territory". The "good diet, good health" path, in Italian "Buona alimentazione", is designed to provide information on how to adopt a healthy diet as a way to promote healthy aging. This is achieved through the organization of cooking courses, workshops, seminars, and thematic dinners. The "Let's work out together" path, in Italian "Muoversi insieme", fosters group exercising by making available to the participants gym classes, walking groups, and martial art courses. The "Surprise and amazement" path (in Italian "Meraviglia e stupore") aims to promote socialization and cultural activity through museum visits, reading experiences, and active theatre and music experiences. The interventions envisaged by this path also seek to make healthcare settings (i.e., hospitals, nursing homes) more human through the introduction of cultural experiences (i.e., art exhibitions, conferences). Lastly, through the "Discovery of the territory" path ("Scoperta del territorio"), our project aims to promote both active life and the discovery of territory by organizing events such as hiking or walking in local forests and natural parks.

The teaching was carried out in the form of meeting and classes. A series of videos available on the Dedalo website (<https://progettodedalo.net/>, www.facebook.com/ProgettoDedaloVercelli/) were also shared with the participants.

Questionnaire and Data Collection

The baseline survey of the longitudinal study was carried out from September to December 2018 using a standardized structured questionnaire. Data were collected by means of the REDCap software (version 9.5.14—© 2021 Vanderbilt University).

The questionnaire investigated the following aspects: (1) socio-demographic data (age, gender, education level, nationality, and the length of their stay in Italy, in the case of foreign nationalities); (2) perception of economic problems; (3) daily diet (fruit and vegetable, sweet drinks, and salt intake); (4) smoking habits and alcohol intake (daily drinking and binge drinking); (5) regular practice of physical exercises—by measuring their intensity levels, duration, and weekly frequency; (6) health status (diseases, medication intake and self-monitoring of blood pressure); (7) anthropometric parameters (height and weight); (8) satisfaction and wellbeing; (9) limitations of daily life due to physical and/or mental conditions; (10) active mobility (bicycle and walking); (11) active life at work; (12) attendance to cultural and social activities; and (13) knowledge of the Dedalo project and participation in its initiatives. The questionnaire (Annex 1) was based on validated questions extracted from the data collection tools of two national surveys, PASSI and Passi D'Argento (Epicentro - ISS, 2019a, 2019b).

In order to improve the response rate of the survey, the interviewers made as many as five call attempts for each candidate, two of which in the evening (after 7:00 pm). Only after five unsuccessful attempts or in case of refusal, the candidate was substituted. Subjects of the sample that were institutionalized (hospital, retirement home, prison) were also replaced with same age and gender candidates. The informed consent was collected at the beginning of each call. The data were stored in compliances with the data confidentiality and in accordance with the Italian legislation (Legislative Decree 196/2003) and the General Data Protection Regulation (EU) 2016/679 (GDPR).

Measurements and Outcomes

Outcomes of the evaluation study were smoking and diet (fruit and vegetables, salt, and sugar drink intake), physical exercise (frequency and duration of moderate and high-level intensity), alcohol intake (daily intake and binge drinking), body mass index (BMI), cognitive activity, socialization, and wellbeing. For this cross-sectional analysis, the outcome was “participation to Dedalo project” and the above variables were studied as behavioral risk or protective factors and psychophysical conditions associated with chance to participate in Dedalo.

The following continuous variables were categorized: *i*) physical activity ($< / \geq 150$ min of moderate physical activity/week *vs* 75 min of intense physical activity/week) (WHO, 2020); *ii*) fruit and vegetable daily intake ($< / \geq 5$ portions/die); *iii*) BMI (< 25 kg/m²; $25\text{--}30$ kg/m²; ≥ 30 kg/m²). Sugar drink consumption was measured with a relative frequency scale and analyzed considering two categories: never or < 1 glass of sugar drinks per week; and > 1 glass of sugar drinks per day.

About smoking and alcohol consumption, the first was classified into non-smoker, current smoker (i.e., smoker for more than 6 months before the interview day), and former smoker. Alcohol intake was also measured with a scale of relative frequency and was investigated with information about daily drinking and binge drinking, with this latter personalized by gender. This led to the following classification: no alcohol intake; 1 alcohol unit per day; 2–3 units per day; and 4 or more units per day.

Two classes encompassing cognitive and socialization activities were established: no active attendance in the past month, one or more listed attendance activities. Physical or psychological illnesses, days of physical inability, days of psychological disabilities were all classified according to the number of days reported: none, from 1 to 5, > 5 days in the last 30 days. The variable “Life satisfaction”, a single item scale with partial semantic autonomy of categories (very little, little, sufficient, highly), was classified in two other sub-groups: “medium-highly satisfied” and “poorly or not at all satisfied”.

Age was analyzed as both continuous and categorical variable, considering 5 age classes: < 50, 50–59, 60–69, and ≥ 70 years old. Three classes of education levels were defined: middle school or less; high school; and University degree. Satisfaction for economic resources was measured with a single item scale with partial semantic autonomy of categories, with four categories ranging from “very little difficulty” to “high difficulty”. This variable was then analyzed using two categories: presence or absence of reported economic difficulties. Finally, two classes of occupation status were defined: “worker” and “retired or other”.

Statistical Analysis

Descriptive statistics of the whole study population or of the two separate groups (people exposed to the Dedalo’s interventions *vs* people not exposed) were carried out.

The categorical variables were reported as absolute number and percentage; the continuous variables were reported as mean, minimum and maximum value, and standard deviation (SD).

To test the differences for the categorical variables between the two groups, the Chi² or Fisher test was performed as appropriate. In order to detect any differences between the continuous variables between the two groups, the normal distribution of the variables was assessed through the Shapiro–Wilk test; consequently, refusing the null hypothesis, the Mann Whitney nonparametric test was performed. The statistical significance was established for a $p\text{-value} \leq 0.05$.

To test the association between the outcome variable “to be in the exposure group” and the studied variables, a univariate regression logistic model was performed. These analyses were described reporting the $p\text{-value}$, the odds ratio (OR) and the confidence interval at 95% (95% CI). Analysis was performed using as reference category the best condition for the participant respect to a healthy and active ageing (lack of risk factors / presence of protective factors). So, for example we analyzed the chance to participate to the Dedalo activities passing from the best conditions (daily intake of ≥ 5 fruit/vegetable portions) to the worsts (not any daily intake

of fruits / vegetables). The multivariate regression logistic analysis was performed in order to adjust the association analyses for gender and age-class.

The statistical analyses were performed using the statistical software Stata MP 13.

Results

People attending any of the Dedalo activities from March to December 2018 amounted to 345, of whom 239 provided a valid telephone number where they could be reached. Of them, 155 (64.9%) completed the baseline interview and were therefore enrolled in the study. The responders of the control group were 214, who came from a larger pool of 856 subjects residing in the municipality of Vercelli randomly extracted (Fig. 1).

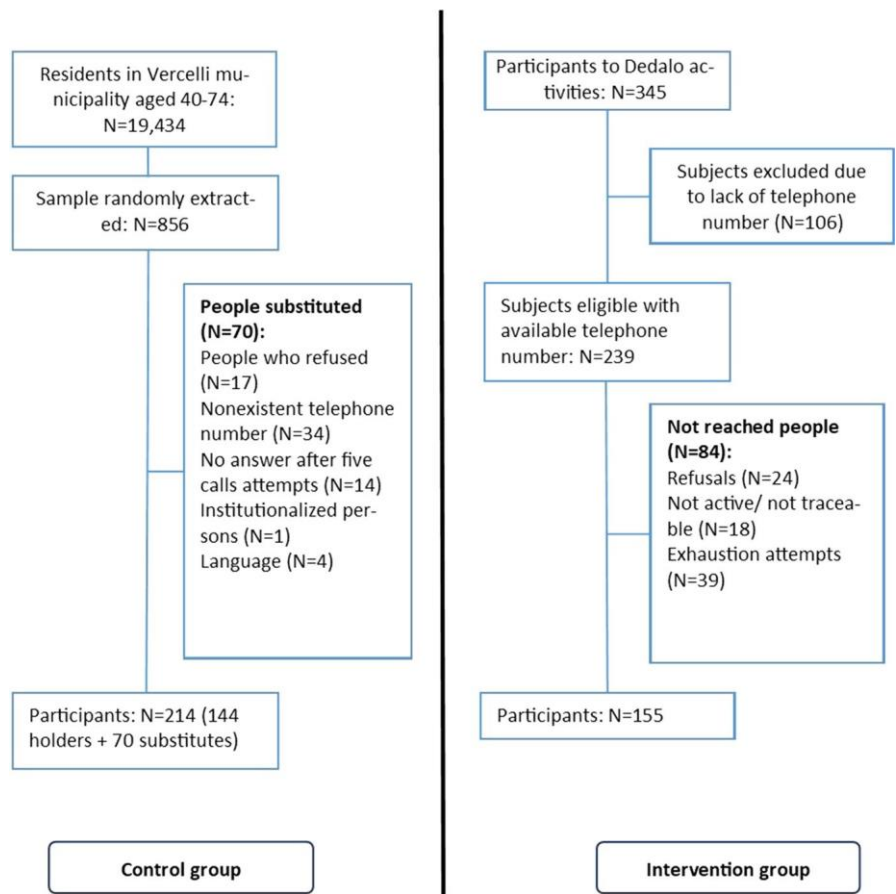


Fig. 1 Flowchart of the study population showing the selection process of participants in the survey

The analyses of the demographic characteristics showed that the two groups under study did not present any statistically significant differences in the mean age (58.97 ± 8.84 vs. 57.59 ± 10.09 in Dedalo participants and control groups, respectively; p -value 0.1647). On the other hand, statistically significant differences emerged for distributions of the following variables in the two groups: age classes, sex, education level, and economic difficulties. Thus, among Dedalo participants 50–69 old-age women with higher educational levels and perceiving lower economic difficulties are more represented than in the control group. By contrast, no differences were found in occupational status (worker vs retired/other, p -value = 0.45) and citizenship (Italy vs. other countries, p -value = 0.48) (Table 1).

Table 2 shows distribution between the two groups of the behavioral risks. Differences between groups were statistically significant for all variables except for BMI. Dedalo participants had self-report higher weekly minutes of moderate physical activity (by contrast, control group declared higher weekly minutes of intense

Table 1 Sociodemographic features of study participants about sex, age, education level, economic difficulties, and occupational status declared during the interviews

	Dedalo participants (%) (n = 155)	Control (%) (n = 214)	Total % (n = 369)	<i>p</i> -value
<i>Sex</i>				
Males	29 (18.7%)	104 (48.6%)	133 (36.3%)	< 0.001 ^b
Females	126 (81.3%)	110 (51.4%)	236 (64.0%)	
<i>Age classes, years</i>				
< 50	27 (17.4%)	58 (27.1%)	85 (23.0%)	< 0.001 ^a
50–59	48 (31.0%)	60 (28.0%)	108 (29.3%)	
60–69	63 (40.7%)	51 (23.8%)	114 (30.9%)	
≥ 70	17 (11.0%)	45 (21.0%)	62 (16.8%)	
<i>Education level</i>				
None/ primary or middle school	23 (14.8%)	66 (30.8%)	89 (24.1%)	< 0.001 ^a
High school	82 (52.9%)	116 (54.2%)	198 (53.7%)	
University degree	50 (32.3%)	32 (15.0%)	82 (22.2%)	
<i>Economic difficulties^c</i>				
Yes	41 (26.6%)	84 (39.2%)	125 (34.0%)	0.012 ^b
No	113 (73.4%)	130 (60.8%)	243 (66.0%)	
<i>Occupational status^d</i>				
Workers	56 (48.7%)	76 (53.9%)	132 (51.6%)	0.451 ^b
Retired and other non-workers	59 (51.3%)	65 (46.1%)	124 (48.4%)	
<i>Citizenship</i>				
Italy	152 (98.7%)	208 (97.2%)	360 (97.8%)	0.477 ^b
Other countries	2 (1.3%)	6 (2.8%)	8 (2.2%)	

^a p -value of Chi-2 test

^b p -value of Fisher's exact test

^cFor this variable, 1 missing data

^dFor this variable, 113 missing data, 40 and 73 in exposure and unexposure groups, respectively

Table 2 Behavioral risk factors distribution among exposure and unexposure groups

Risk factors	Dedalo participants (%) (n = 155)	Controls (%) (n = 214)	Total (%) (n = 369)	<i>p</i> -value
<i>BMI</i>				
< 25	92 (59.4%)	106 (49.5%)	198 (53.7%)	0.157 ^a
25 – 29	47 (30.3%)	77 (36.0%)	124 (33.6%)	
≥ 30	16 (10.3%)	31 (14.5%)	47 (12.7%)	
<i>Fruit/vegetable portions</i>				
≥ 5	33 (21.3%)	17 (7.9%)	50 (13.5%)	< 0.001 ^b
< 5	122 (78.7%)	197 (92.1%)	319 (86.5%)	
<i>Smoke</i>				
Never	101 (65.6%)	99 (46.2%)	200 (54.4%)	< 0.001 ^a
Ex-smoker	45 (29.2%)	59 (27.6%)	104 (28.3%)	
Current smoker	8 (5.2%)	56 (26.2%)	64 (17.4%)	
<i>Minutes/week of moderate physical activity</i>				
≥ 150	59 (40.0%)	62 (25.6%)	121 (32.8%)	0.014 ^b
< 150	155 (60.0%)	93 (72.4%)	248 (67.2%)	
<i>Minutes/week of intense physical activity</i>				
≥ 75	14 (9.0%)	35 (16.4%)	49 (13.3%)	0.044 ^b
< 75	141 (91.0%)	179 (83.6%)	320 (86.7%)	
<i>Salt intake attention</i>				
Yes	129 (83.2%)	155 (72.4%)	284 (77.0%)	0.017 ^b
No	26 (16.8%)	59 (27.6%)	85 (23.0%)	
<i>Consumption of sugar drinks, glasses/week</i>				
Never	133 (85.8%)	137 (64.0%)	270 (73.2%)	< 0.001 ^b
1 or more	22 (14.2%)	77 (36.0%)	99 (26.8%)	
<i>One or more cognitive/socialization activities in the last 30 days</i>				
Yes	149 (96.1%)	189 (88.3%)	338 (91.6%)	0.007 ^b
No	6 (3.9%)	25 (11.7%)	31 (8.4%)	
<i>Alcohol intake, glasses/day</i>				
Never	35 (22.9%)	66 (30.8%)	101 (27.5%)	0.042 ^a
≤ 1	103 (67.3%)	125 (58.4%)	228 (62.1%)	
2–3	12 (7.8%)	23 (10.8%)	35 (9.5%)	
4 or more	3 (2.0%)	0 (0.0%)	3 (0.8%)	

^ap-value of Chi-2 test^bp-value of Fisher's exact test

physical activity), major attention in salt intake, none sugar drink consumption, less daily alcohol consumption, and more social activities participation in comparison to the control. In addition, the exposure group had self-report most often never smoke habits respect to the unexposure one (65.6% vs. 46.2%, respectively) (see Table 2).

Psychophysical conditions are showed in Table 3. Differences between groups emerged for *Unhealthy days* for psychological conditions, life satisfaction, diagnosis of myocardial infarction, cancer, and arthrosis. Thus, in comparison to control

Table 3 Psychophysical conditions distribution among exposure and unexposure groups

Psychophysical conditions	Dedalo participants % (N = 155)	Controls % (N=214)	Tot. % (N=369)	<i>p</i> -value
<i>Life satisfaction</i> ^c				
Medium–high	144 (93.5%)	169 (79.3%)	313 (85.3%)	< 0.001 ^b
Low	10 (6.5%)	44 (20.7%)	54 (14.7%)	
<i>Physical illness, No. of unhealthy days in the last month</i>				
0	96 (61.9%)	133 (62.2%)	229 (62.1%)	0.459 ^a
≤ 5	34 (21.9%)	55 (25.7%)	89 (24.1%)	
> 5	25 (16.2%)	26 (12.2%)	51 (13.8%)	
<i>Psychological conditions, No. of unhealthy days in the last month</i>				
0	77 (49.7%)	148 (69.2%)	225 (61.0%)	< 0.001 ^a
≤ 5	39 (25.2%)	26 (12.2%)	65 (17.6%)	
> 5	39 (25.2%)	40 (18.7%)	79 (21.4%)	
<i>Self-reported diseases at least one</i>				
No	68 (43.9%)	80 (37.4%)	148 (40.1%)	0.237 ^b
Yes	87 (56.1%)	134 (62.6%)	221 (59.9%)	
<i>Self-reported diseases</i> ^b				
Diabetes	6 (3.9%)	11 (5.1%)	17 (4.6%)	0.624
Respiratory illness	6 (3.9%)	15 (7.0%)	21 (5.7%)	0.257
Hypertension	45 (29.0%)	70 (32.7%)	115 (31.2%)	0.495
Myocardial infarction	2 (1.3%)	13 (6.1%)	15 (4.1%)	0.030
Other heart diseases	14 (9.0%)	9 (4.2%)	23 (6.2%)	0.080
Ictus	2 (1.3%)	4 (1.9%)	6 (1.6%)	1.000
Liver diseases	3 (1.9%)	4 (1.9%)	7 (1.9%)	1.000
Cancer	26 (16.8%)	19 (8.9%)	45 (12.2%)	0.025
Arthrosis	36 (23.2%)	77 (36.0%)	113 (30.6%)	0.009
<i>Treated hypertension</i>				
No	115 (74.2%)	145 (67.8%)	260 (70.5%)	0.204 ^b
Yes	40 (25.8%)	69 (32.2%)	109 (29.5%)	

^ap-value of Chi-2 test

^bp-value for Fisher’s exact test

^cFor this variable, 2 missing data

group, Dedalo participants self-reported most often a medium–high level of life satisfaction, a higher number of unhealthy days for psychological conditions, less diagnosis of myocardial infarction, arthrosis but more diagnosis of cancer (see Table 3).

Table 4 shows the chance to participate to the Dedalo’s activities passing from the best conditions, that is characterized by lack of risk factors / presence of protective factors (i.e., daily intake of ≥ 5 fruit/vegetable portions, BMI < 25, etc.), to the worsts (i.e., not any daily intake of fruits and vegetables, BMI ≥ 30 , etc.). Participants in the Dedalo project were less exposed to all the analyzed risk factors. In the model adjusted by sex and age class, they were significantly less exposed to a low consumption of fruits and vegetables, to current smoking (OR = 0.20, CI

Table 4 Risk factor distribution for exposure and unexposure groups analyzed in the evaluation study of the Dedalo project: unadjusted and adjusted (by sex and age classes) logistic models for the chance to participate to Dedalo

Risk factors	Dedalo participants % (n = 155)	Controls % (n = 214)	OR unadjusted, CI 95%	OR adjusted ^a , CI 95%
<i>BMI</i>				
< 25	92 (59.4%)	106 (49.5%)	1	1
25—29	47 (30.3%)	77 (36.0%)	0.70 (0.45–1.11)	1.00 (0.59–1.68)
≥ 30	16 (10.3%)	31 (14.5%)	0.60 (0.31–1.16)	0.81 (0.38–1.72)
<i>Fruit/vegetable portions</i>				
≥ 5	33 (21.3%)	17 (7.9%)	1	1
< 5	122 (78.7%)	197 (92.1%)	0.32 (0.17–0.60)	0.32 (0.16–0.64)
<i>Smoke</i>				
Never	101 (65.6%)	99 (46.2%)	1	1
Ex-smoker	45 (29.2%)	59 (27.6%)	0.75 (0.46–1.20)	1.20 (0.67–2.13)
Current smoker	8 (5.2%)	56 (26.2%)	0.14 (0.06–0.31)	0.20 (0.08–0.46)
<i>Minutes/week of moderate physical activity</i>				
≥ 150	59 (40.0%)	62 (25.6%)	1	1
< 150	155 (60.0%)	93 (72.4%)	0.57 (0.37–0.89)	0.77 (0.47–1.26)
<i>Minutes/week of intense physical activity</i>				
≥ 75	14 (9.0%)	35 (16.4%)	1	1
< 75	141 (91.0%)	179 (83.6%)	1.97 (1.02, 3.80)	1.53 (0.72–3.23)
<i>Salt intake attention</i>				
Yes	129 (83.2%)	155 (72.4%)	1	1
No	26 (16.8%)	59 (27.6%)	0.53 (0.32–0.89)	0.63 (0.35–1.12)
<i>Consumption of sugar drinks, glasses/week</i>				
0	133 (85.8%)	137 (64.0%)	1	1
1 or more	22 (14.2%)	77 (36.0%)	0.29 (0.17–0.50)	0.36 (0.20–0.64)

Table 4 (continued)

Risk factors	Dedalo participants % (n = 155)	Controls % (n = 214)	OR unadjusted, CI 95%	OR adjusted ^a , CI 95%
<i>One or more cognitive/socialization activities in the last 30 days</i>				
Yes	149 (96.1%)	189 (88.3%)	1	1
No	6 (3.9%)	25 (11.7%)	0.30 (0.12–0.76)	0.40 (0.15–1.07)
<i>Alcohol intake, glasses/day</i>				
Never	35 (22.9%)	66 (30.8%)	1	1
≤ 1	103 (67.3%)	125 (58.4%)	1.55 (0.96–2.53)	1.60 (0.92–2.79)
2–3	12 (7.8%)	23 (10.8%)	0.98 (0.44–2.21)	1.61 (0.62–4.19)
4 or more	3 (2.0%)	0 (0.0%)	–	–

95% CI are statistically significant for lower fruits and vegetables intake (<5 daily portions), smoking habits (current smokers), sugar intake (one or more sugar drink glasses per week), socialization habits (no socialization activities in the last 30 days)

CI confidence interval, OR odds ratio

^aModels were adjusted for sex and age classes

Table 5 Psychophysical conditions of the population observed in the evaluation study of the Dedalo project: unadjusted and adjusted (by sex and age classes) logistic models for the chance to participate to Dedalo

Psychophysical conditions	Dedalo participants % (N = 155)	Controls % (N = 214)	OR unadjusted, CI 95%	OR adjusted ^a , CI 95%
<i>Life satisfaction^b</i>				
Medium–high	144 (93.5%)	169 (79.3%)	1	1
Low	10 (6.5%)	44 (20.7%)	0.27 (0.13–0.55)	0.27 (0.12–0.60)
<i>Physical illness, No. of unhealthy days in the last month</i>				
0	96 (61.9%)	133 (62.2%)	1	1
≤ 5	34 (21.9%)	55 (25.7%)	0.86 (0.52–1.41)	0.69 (0.39–1.24)
> 5	25 (16.2%)	26 (12.2%)	1.33 (0.73–2.45)	1.11 (0.55–2.25)
<i>Psychological conditions, No. of unhealthy days in the last month</i>				
0	77 (49.7%)	148 (69.2%)	1	1
≤ 5	39 (25.2%)	26 (12.2%)	2.88 (1.63–5.09)	1.88 (1.01–3.51)
> 5	39 (25.2%)	40 (18.7%)	1.87 (1.11–3.15)	1.40 (0.78–2.54)
<i>Self-reported diseases</i>				
Diabetes	6 (3.9%)	11 (5.1%)	0.74 (0.27–2.06)	0.99 (0.32–3.01)
Respiratory illness	6 (3.9%)	15 (7.0%)	0.53 (0.20–1.41)	0.73 (0.25–2.16)
Hypertension	45 (29.0%)	70 (32.7%)	0.84 (0.54–1.32)	0.67 (0.40–1.12)
Myocardial infarction	2 (1.3%)	13 (6.1%)	0.20 (0.05–0.91)	0.17 (0.03–0.88)
Other heart diseases	14 (9.0%)	9 (4.2%)	2.26 (0.95–5.37)	3.17 (1.15–8.69)
Ictus	2 (1.3%)	4 (1.9%)	0.69 (0.12–3.80)	0.42 (0.07–2.67)
Liver diseases	3 (1.9%)	4 (1.9%)	1.04 (0.23–4.70)	1.78 (0.30–10.41)
Cancer	26 (16.8%)	19 (8.9%)	2.07 (1.10–3.89)	1.78 (0.86–3.69)
Arthrosis	36 (23.2%)	77 (36.0%)	0.54 (0.34–0.86)	0.32 (0.18–0.57)
<i>Self-reported diseases at least one</i>				
No	68 (43.9%)	80 (37.4%)	1	1

Table 5 (continued)

Psychophysical conditions	Dedalo participants % (N= 155)	Controls % (N=214)	OR unadjusted, CI 95%	OR adjusted ^a , CI 95%
Yes	87 (56.1%)	134 (62.6%)	0.75 (0.50–1.16)	0.52 (0.31–0.87)
<i>Treated hypertension</i>				
No	115 (74.2%)	145 (67.8%)	1	1
Yes	40 (25.8%)	69 (32.2%)	0.73 (0.46, 1.16)	0.59 (0.34, 1.00)

95% CI are statistically significant for lower life satisfaction, few unhealthy days in the last month for psychological conditions, people affected by myocardial infarction, other heart diseases, and arthrosis, self-reported at least a diagnosticated disease, people declared drug intake for hypertension

Tot total, *CI* confidence interval, *OR* odds ratio

^aModels were adjusted for sex and age classes

^bFor this variable, 2 missing data

95% 0.08–0.46), and consumption of sugar drinks. No association emerged for alcohol consumption and physical activity in the adjusted models.

Considering the inferential analysis results, the participants in the Dedalo project reported a lower chance of referring heart attacks and arthrosis or a treatment for hypertension, but higher risk of have diagnosis heart diseases (Table 5). Considering quality of life, no differences emerged in unhealthy days for physical illness, whereas Dedalo participants showed a worst psychological status with 88% of chance higher than control to had experience at least 5 unhealthy days for mental distress in the last month. Thus, even though the unexposure group resulted have higher self-reported psychological wellness compared to exposure one, the percentage of individuals reporting high levels of “life satisfaction” was higher

Table 6 Behavioral risk factors and psychophysical conditions of the population observed in the evaluation study of the Dedalo project: adjusted (by sex and age classes) logistic multivariate model for the chance to participate to Dedalo

	Dedalo participants % (N = 155)	Controls % (N=214)	OR adjusted ^a , CI 95%
<i>Smoke</i>			
Never	101 (65.6%)	99 (46.2%)	1
Ex-smoker	45 (29.2%)	59 (27.6%)	1.13 (0.63–2.07)
Current smoker	8 (5.2%)	56 (26.2%)	0.19 (0.08–0.45)
<i>Consumption of sugar drinks, glasses/week</i>			
0	133 (85.8%)	137 (64.0%)	1
1 or more	22 (14.2%)	77 (36.0%)	0.41 (0.22–0.75)
<i>Life satisfaction^b</i>			
Medium–high	144 (93.5%)	169 (79.3%)	1
Low	10 (6.5%)	44 (20.7%)	0.22 (0.09–0.51)
<i>Psychological conditions, No. of unhealthy days in the last month</i>			
0	77 (49.7%)	148 (69.2%)	1
≤ 5	39 (25.2%)	26 (12.2%)	2.59 (1.34–5.00)
> 5	39 (25.2%)	40 (18.7%)	2.01 (1.02–3.97)
<i>Self-reported diseases</i>			
Myocardial infarction	2 (1.3%)	13 (6.1%)	0.18 (0.03–0.94)
Other heart diseases	14 (9.0%)	9 (4.2%)	1.79 (0.61–5.28)

95% CI are statistically significant for smoking habits (current smokers), sugar intake (one or more sugar drink glasses per week), lower life satisfaction, number of unhealthy days in the last month for psychological conditions, self-reported diseases (myocardial infarction)

CI confidence interval, OR odds ratio

^aModel was adjusted for sex and age classes and include the following variables, with low collinearity: smoke habits, sugar drinks consumption, life satisfaction, unhealthy days for mental distress, diagnosed myocardial infarction and heart diseases

^b Life satisfaction was measured with a 5-points frequency item from "highly" to "very little" (very little, little, sufficient, highly). The first two points were categorized in low satisfaction, whereas the last two in medium-high satisfaction

in Dedalo group than the control: passing from the best condition (medium–high level of life satisfaction) to the worst (low level) the chance to not attendance to Dedalo activities increase (73% higher compared to control in the both adjusted and unadjusted models, Table 5). Furthermore, the exposure individuals were generally healthier, with 43.9% of them not reporting any of the investigated diseases vs 37.4% of controls.

Finally, Table 6 presents results of an adjusted (by sex and age) multivariate logistic regression including behavioral risk factors (smoke habits, sugar drinks consumption) and psychophysical conditions (life satisfaction, unhealthy days for mental distress, diagnosis of myocardial infarction, and heart disease) associated to the outcome in the adjusted models by sex and age (Tables 4 and 5).

Thus, all associations were confirmed except for self-reported diagnosis of “Other heart diseases” and ex-smokers. Among factors associated to participation, we can note a considerable increase in ORs related to unhealthy days for mental distress (ORs 2.59 95% CI 1.34–5.00 and 2.01 95% CI 1.02–3.97 for ≤ 5 and > 5 unhealthy days for mental distress, respectively) (Table 6).

Discussion

The purpose of this study is to assess the ability of the Dedalo project, a multicomponent community intervention for the promotion of healthy aging, to enroll the target population avoiding selection in terms of SES. This was done by using the baseline data of the longitudinal study aimed to evaluate the impact of the intervention on the main outcomes of the intervention. The project, implemented in Vercelli, a medium-small city in the North of Italy, consists of different types of interventions encompassing the following four themes: healthy diet, physical exercise, socialization and culture, and discovery of the local territory.

Our results suggest that the activities promoted by the Dedalo project are predominantly attended by women (81.3%) with a high education level and low economic difficulties, compared to unexposure controls randomly selected from the general population. The participants also tend to have healthier behaviors compared to the general population: they smoke rarely, consume a large amount of fruit and vegetables, and have a low intake of sugar drinks.

Another interesting observation is that people involved in the Dedalo’s interventions tend to be healthier than control subjects, except for the variable “other hearth diseases”. These characteristics are consistent with those observed in volunteers taking part in preventive interventions and health promotion activities within the community (Chylińska et al., 2017; Marmot & Bell, 2019; WHO, 2010). This is particularly true in Italy, where social behaviors, such as participation in community services, are inversely associated with education levels, economic difficulties, and age (Epicentro - ISS, 2019b). Our data are also in good agreement with previously published studies showing social inequalities in health (Marmot & Bell, 2019; WHO, 2010).

Dedalo project was also characterized by overwhelming attendance of women to its activities. This is not surprising given that older women in Italy are typically

more engaged in social activities requiring voluntary work (elderly people as “resource” for family, community, and economy (WHO, 2002))—e.g., family or community caregiving initiatives—than men (32.4% of women vs. 23.7% of men), who instead participate more than women (23% and 21%, respectively) to community and cultural activities and events—e.g., community life, travels, and training activities (Epicentro - ISS, 2019b).

A last observation is worth to be done: the percentage of smokers in the population adhering to the project is one fifth of that of the control group and of the general Italian adult population (Epicentro - ISS, 2019a). Considering that people from lower SES groups are more likely to smoke and less likely to quit smoking than those from higher SES groups (Brown et al., 2014), our results appear to indicate a severe selection in the enrollment of the target population.

Altogether, the previous observations show that Dedalo project attracted a selected population, characterized to be of higher social class, to have a lower level of exposure to risk factors, and a better health and wellbeing, predominantly female. The findings herein reported show that the Dedalo project has so far failed to reach one of its primary objectives: the reduction of social inequalities in health.

Indeed, the observation that the vast majority of unhealthy behaviors were less frequent among the program participants compared to a random sample of the general population suggests that the individuals enrolled in the initiative were probably those in less need of preventive interventions. Assuming that socioeconomic status of the local population is distributed like the mean of the population of the Northern

Italy, we hypothesize for Vercelli’s municipality that among people with 40 years old or more, about 45% have low educational levels and about the 7% (≥ 35 years old) are in relative poverty (Istat 2020a, 2020b). Considering the amount of population at risk (for SES), it is imperative that we identify alternative enrollment strategies capable of targeting people with lower SES and adult men, who at the moment do not seem to be particularly attracted by the program.

One possible explanation for this failure is that the communication channels that were used to advertise the various initiatives (e.g., fliers, newspaper ads, websites, social networks, and word of mouth) may have caused a selection bias, excluding the people most in need (i.e., lower SES subjects and adult men).

In this regard, a review on social marketing for behavior change has underscored several tools with which to achieve a more effective advertising for these types of initiatives (McKenzie-Mohr & Schultz, 2014). For instance, the promotion of healthy behaviors based on local social norms focusing on the social identity of the community or the use of a renowned influencer has proven highly effective in increasing the motivation levels of the target population to participate in an intervention.

The results of this study have been used to change the communication approach of the Dedalo activities, including more local channels in order to reach people in their neighborhoods and involving local associations in the conduction of the intervention. Furthermore, although Dedalo is a program for general population, its events and activities can be addressed also to a specific target population that could have great benefits with participation. For example, a walking group in Dedalo was planned for patients of Addiction Service, an “emotion artistic workshop” for oncologic patients, or dance therapy course for patients with Parkinson disease.

This cross-sectional analysis has a limit in the method used in the sampling. First, the type of variables used as *proxy* of SES indicator: educational level and economic difficulties perceived by participants. This last variable is very easy to measure with low risk of bias for *social desirability*, but is strongly subjective. So, to consider social inequality in the preventive intervention access, it would be recommended to use more objective indicators to measure SES status. Lastly, for the type of sampling stratification (by age and sex) of the randomized control, that likely have led to a low presence of people with foreign origin among unexposure group (about 3% of unexposure participants vs. about 7% of the total Vercelli's residents with 40 years and more) (Istat, 2019).

Another possible limit is that the Dedalo activities are carried out in Italian, with the risk to exclude foreign citizens. The percentage of foreign people living in Vercelli is quite low and about 6,7% of the total residents (in the age class > 40 years, in 2019); moreover national data showed that in Italy about 64% of the foreign people have not any difficulty to understand Italian (Istat, 2019). Thus, this possible bias seems to have a low impact.

Conclusion

This study is one of the first evaluations of a community intervention in Italy and, to our knowledge, in Europe. Our findings indicate that the Dedalo project has so far been unsuccessful in engaging in its initiatives the most vulnerable population to NCDs, thereby failing to meet the goal of reducing health disparities.

Even though our results are essentially negative, we strongly believe that they will provide a useful source of information to better understand the impact of preventive interventions on health inequalities within a community, thereby fostering future collaborative research endeavors.

Acknowledgements We thank the ASL (Local Health Authority) of Vercelli for its contribution in the conduction of the study and the Dedalo project.

Author contributions Project administration (Fabrizio Faggiano); Funding acquisition (Fabrizio Faggiano); Conceptualization (Alessandro Coppo, Fabrizio Faggiano); Methodology (Sara Bortoluzzi, Alessandro Coppo, Chiara Airoidi, Fabrizio Faggiano); Investigation (Sara Bortoluzzi, Alessandro Coppo, Stefano Parovina, Sara Napoletano, Irene Ammazzagatti, Angelica Zibetti, Chiara Aleni); Formal analysis (Sara Bortoluzzi, Chiara Airoidi); Data curation (Chiara Airoidi); Software (Chiara Airoidi); Writing—Original Draft (Sara Bortoluzzi); Writing—Review and Editing (Silvia Caristia, Daniela Alessi, Chiara Airoidi, Fabrizio Faggiano); Visualization (Silvia Caristia); Project implementation and Resources (Dedalo Coordinating group).

Funding Open access funding provided by Università degli Studi del Piemonte Orientale Amedeo Avogadro within the CRUI-CARE Agreement. The evaluation of DEDALO Project is partially funded by the AGING Project of the Department of Transational Medicine of the Università del Piemonte Orientale (“MIUR Dipartimenti di Eccellenza 2018–2022”), and by a Grant from the Fondazione Cassa di Risparmio di Vercelli. Sponsors were not involved in any phases of the study.

Data availability Due to the sensitive nature of the questions asked in this study, survey, respondents were assured that the raw data would remain confidential and would not be shared with third parties.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article. Dr. Fabrizio Faggiano is an Associated Editor of the Journal.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. The ethical approval was obtained from the Ethics Committee of Alessandria “Comitato Etico Interaziendale ASO SS. Antonio e Biagio e C. Arrigo” (No. AslVC.Med.18.02). The protocol of the study is registered at ClinicalTrial.gov (clinical trial registration No. NCT03939754).

Informed Consent Informed consent was obtained from all individual participants included in the study.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- ASL VC (2021) Dedalo—Volare sugli anni. http://www.aslvc.piemonte.it/index.php?option=com_content&view=article&id=864:dedalo-volare-sugli-anni&catid=63:ambito-della-prevenzione. Accessed 14 Oct 2021.
- Benziger, C. P., Roth, G. A., & Moran, A. E. (2016). The Global Burden of Disease Study and the Preventable Burden of NCD. *Global Heart*, 11, 393–397.
- Brown, T., Platt, S., & Amos, A. (2014). Equity impact of European individual-level smoking cessation interventions to reduce smoking in adults: A systematic review. *European Journal of Public Health*, 24, 551–556. <https://doi.org/10.1093/eurpub/cku065>
- Chylińska, J., Łazarewicz, M., Rzakiewicz, M., et al. (2017). The role of gender in the active attitude toward treatment and health among older patients in primary health care-self-assessed health status and sociodemographic factors as moderators. *BMC Geriatrics*, 17, 284. <https://doi.org/10.1186/s12877-017-0677-z>
- Epicentro—ISS (2019a). Sorveglianza PASSI. <https://www.epicentro.iss.it/passi/dati/attivita?tab-container-1=tab1#nazionali>. Accessed 20 Jul 2021.
- Epicentro—ISS (2019b). Sorveglianza Passi d’Argento. <https://www.epicentro.iss.it/passi-argento/>. Accessed 20 Jul 2021.
- Gakidou, E., Afshin, A., Abajobir, A. A., et al. (2017). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, 390, 1345–1422. [https://doi.org/10.1016/S0140-6736\(17\)32366-8](https://doi.org/10.1016/S0140-6736(17)32366-8)
- Gottfredson, D. C., Cook, T. D., Gardner, F. E. M., et al. (2015). Standards of evidence for efficacy, effectiveness, and scale-up research in prevention science: Next Generation. *Prevention Science*, 16, 893–926. <https://doi.org/10.1007/s1121-015-0555-x>
- Istat. (2021). Le condizioni di salute della popolazione anziana in Italia. Anno 2019. Roma.
- Istat (2018) Indicatori demografici. Stime per l’anno 2017. 1–15
- Istat. (2020a). I.Stat. In: Pop. e Fam. <https://dati.istat.it>. Accessed 26 Jan 2021.
- Istat. (2020b). I.Stat. In: Condizioni Econ. delle Fam. e disuguaglianze. <http://dati.istat.it>. Accessed 4 May 2022.
- Istat. (2019). Istat. In: Demogr. cifre. <https://demo.istat.it/>. Accessed 6 May 2022.
- Marmot, M., & Bell, R. (2019). Social determinants and non-communicable diseases: Time for integrated action. *BMJ*, 364, 10–12. <https://doi.org/10.1136/bmj.1251>

- McKenzie-Mohr, D., & Schultz, P. W. (2014). Choosing effective behavior change tools. *Soc Mar Q*, 20, 35–46. <https://doi.org/10.1177/1524500413519257>
- Ministero della Salute—Direzione Generale della Prevenzione Sanitaria. (2020). Piano Nazionale della Prevenzione 2020–2025. Roma.
- Murray, C. J. L., Aravkin, A. Y., Zheng, P., et al. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet*, 396, 1223–1249. [https://doi.org/10.1016/S0140-6736\(20\)30752-2](https://doi.org/10.1016/S0140-6736(20)30752-2)
- Skolnik, R. (2018). Noncommunicable Diseases Country Profiles 2018.
- Vos, T., Lim, S. S., Abbafati, C., et al. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet*, 396, 1204–1222. [https://doi.org/10.1016/S0140-6736\(20\)30925-9](https://doi.org/10.1016/S0140-6736(20)30925-9)
- WHO. (2011). Global status report on noncommunicable diseases 2010. Geneva.
- WHO. (2010). Western Pacific Region. Noncommunicable disease risk factors and socioeconomic inequalities—what are the links? A multicountry analysis of noncommunicable disease surveillance data Report to the WHO Regional Office for the Western Pacific Section.
- WHO. (2020). WHO Guidelines on physical activity and sedentary behaviour. Geneva.
- WHO. (2002). Active ageing. A policy framework. Geneva.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Sara Bortoluzzi¹ · Alessandro Coppo² · Daniela Alessi² · Stefano Parovina³ · Sara Napoletano³ · Irene Ammazzagatti³ · Chiara Airoidi³ · Angelica Zibetti⁴ · Chiara Aleni³ · Silvia Caristia³  · Fabrizio Faggiano^{2,3} · DEDALO Coordinating Group

✉ Silvia Caristia
silvia.caristia@uniupo.it

¹ School of Hygiene and Preventive Medicine, Department of Translational Medicine, Università degli Studi del Piemonte Orientale, Via Solaroli 17, 28100 Novara, Italy

² Epidemiologic Unit, Vercelli Local Health Authority, Largo Giuseppe Giusti 13, 13100 Vercelli, Italy

³ Department of Translational Medicine, Università del Piemonte Orientale, Via Solaroli 17, 28100 Novara, Italy

⁴ Institute of Legal Medicine, Università “Magna Grecia” di Catanzaro, Catanzaro, Italy



Systematic Review

Using Ambulatory Care Sensitive Conditions to Assess Primary Health Care Performance during Disasters: A Systematic Review

Alessandro Lamberti-Castronuovo ^{1,2,*}, Martina Valente ^{1,2}, Chiara Aleni ², Ives Hubloue ³,
Luca Ragazzoni ^{1,2} and Francesco Barone-Adesi ^{1,4}

- ¹ CRIMEDIM—Center for Research and Training in Disaster Medicine, Humanitarian Aid and Global Health, Università del Piemonte Orientale, 28100 Novara, Italy; martina.valente@uniupo.it (M.V.); luca.ragazzoni@med.uniupo.it (L.R.); francesco.baroneadesi@uniupo.it (F.B.-A.)
 - ² Department for Sustainable Development and Ecological Transition, Università del Piemonte Orientale, 13100 Vercelli, Italy; chiara.aleni@uniupo.it
 - ³ Research Group on Emergency and Disaster Medicine, Vrije Universiteit Brussel, 1050 Brussels, Belgium; ives.hubloue@vub.be
 - ⁴ Department of Translational Medicine, Università del Piemonte Orientale, 28100 Novara, Italy
- * Correspondence: alessandro.lamberti@uniupo.it

Abstract: Ambulatory care sensitive conditions (ACSCs) are health conditions for which appropriate primary care intervention could prevent hospital admission. ACSC hospitalization rates are a well-established parameter for assessing the performance of primary health care (PHC). Although this indicator has been extensively used to monitor the performance of PHC systems in peacetime, its consideration during disasters has been neglected. The World Health Organization (WHO) has acknowledged the importance of PHC in guaranteeing continuity of care during and after a disaster for avoiding negative health outcomes. We conducted a systematic review to evaluate the extent and nature of research activity on the use of ACSCs during disasters, with an eye toward finding innovative ways to assess the level of PHC function at times of crisis. Online databases were searched to identify papers. A final list of nine publications was retrieved. The analysis of the reviewed articles confirmed that ACSCs can serve as a useful indicator of PHC performance during disasters, with several caveats that must be considered. The reviewed articles cover several disaster scenarios and a wide variety of methodologies showing the connection between ACSCs and health system performance. The strengths and weaknesses of using different methodologies are explored and recommendations are given for using ACSCs to assess PHC performance during disasters.

Keywords: ambulatory care sensitive conditions; avoidable hospitalizations; disasters; primary health care; vulnerable populations; H-EDRM



Citation: Lamberti-Castronuovo, A.; Valente, M.; Aleni, C.; Hubloue, I.; Ragazzoni, L.; Barone-Adesi, F. Using Ambulatory Care Sensitive Conditions to Assess Primary Health Care Performance during Disasters: A Systematic Review. *Int. J. Environ. Res. Public Health* **2022**, *19*, 9193. <https://doi.org/10.3390/ijerph19159193>

Academic Editor: Paul B. Tchounwou

Received: 24 June 2022

Accepted: 25 July 2022

Published: 27 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

In recent years, a new common and comprehensive approach to disaster management (DM) has emerged, which focuses on prevention and preparedness strategies. This new approach promotes coordination across an entire health system, as well as between the health system and other sectors [1]. In 2019, the World Health Organization (WHO) published the Health Emergency and Disaster Risk Management (H-EDRM) framework, detailing how this comprehensive approach can be implemented by stakeholders in all sectors working to reduce health risks and consequences of disasters [2]. In particular, the H-EDRM framework's "whole-of-health-system" approach to DM stresses the need for each level of a health care system to support disaster preparedness, including the primary health care (PHC) system.

The PHC system can take on a variety of crucial roles during a disaster [3]. It can be the first point of entry to health care, addressing the health needs and priorities of vulnerable

and marginalized groups who usually bear the worst consequences of a disaster. It can also prevent the overburdening of hospital and emergency services by maintaining continuity of care for those with minor injuries and non-emergent conditions. Lastly, it can reduce the severity of the surge in demand for chronic care needs in the extended period following the acute response [4].

Hospitalization rates for ambulatory care sensitive conditions (ACSCs) have proven to be a reliable proxy for measuring the quality and accessibility of PHC systems [5–8]. The concept of ACSCs was first introduced in the late 1980s by John Billings of New York University as a means for describing the differences in access and utilization of health services among vulnerable populations [9]. According to Billings' definition, ACSCs are "health conditions-diagnoses for which timely and effective outpatient care can help reduce the risks of hospitalization by either preventing the onset of an illness or condition, controlling an acute episodic illness or condition, or managing a chronic disease". Elevated ACSC hospitalization rates suggest that there are problems in access to care, especially for disadvantaged and marginalized populations. Surveillance of hospital admissions for these conditions may thus be used as a population-based indicator both to evaluate and compare the quality and utilization of PHC and to identify subgroups not receiving adequate and timely access to PHC [10,11].

The literature on ACSCs has been growing over the past ten years. Besides methodological publications aimed at defining country-specific lists of ACSCs [12] and those unraveling methodological implications and potential pitfalls deriving from the use of ACSCs [13], the published original studies have primarily aimed at comparing hospitalization rates due to ACSCs across different groups or countries [14–16], performing economic evaluations of avoidable hospitalizations [17,18], identifying inequalities in terms of access to ambulatory care [19,20], and examining trends over the years to spot temporal increases and geographic variations [21,22]. Despite the vast body of literature using ACSC hospitalizations as an indicator of PHC performance and functioning, the literature examining ACSC hospitalizations during disasters and public health emergencies is scarce.

Measuring the performance of a PHC system during different phases of a disaster can contribute to building health systems that are more effective and resilient through all stages of DM. Although ACSC indicators are useful for understanding PHC performance, their use in disaster epidemiology has not been thoroughly examined. As of yet, there is no clear indication of how such indicators can be used to advance this field of research. The aim of this review is to understand to what extent ACSC hospitalization rates during disasters have been studied, and how accurately they can be used to assess PHC performance.

2. Materials and Methods

2.1. Search Strategy and Eligibility Criteria

This review was conducted in January 2022, following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) checklist. The PubMed, Web of Science, and Scopus databases were searched to find relevant publications. The following were the main eligibility criteria for articles in the study: (i) the study measures ACSC hospitalizations during a disaster, either to assess PHC performance or not; (ii) the study indicates the list of ACSC diagnoses that were considered; and (iii) the study is written in English, Italian, Portuguese, French, German, Arabic, or Romanian. Articles were therefore excluded whenever they focused on ED encounters/calls rather than hospitalizations, targeted non-disaster situations, or did not report a list of ACSC diagnoses. In order to obtain a comprehensive overview of the subject, no restrictions were applied regarding the study type or year of publication. Titles, abstracts, and, when applicable, MeSH terms were searched by combining two groups of key words, namely, ACSC- and disaster-related keywords, using Boolean operators AND and OR (Supplementary File S1). The search and screening of titles and abstracts were performed by authors ALC, CA, and MV, against the agreed inclusion and exclusion criteria; disagreements between reviewers were resolved by consensus. Because of the surge in publications on health system performance during

the COVID-19 pandemic, a second search was performed in April 2022, to account for potential new publications measuring ACSCs during the pandemic. This second search was performed using the same search query and databases but restricting the search to 2022 publications.

2.2. Data Extraction and Synthesis

Data were extracted from the retrieved articles and recorded into an extraction sheet (Supplementary File S2). Extracted information was analyzed to evaluate the extent, scope, and nature of available evidence on the use of ACSC indicators during disasters.

3. Results

A total of 1608 records were identified from the database search. Duplicates ($n = 846$) were removed, leaving 762 articles to be judged for relevance. Of those, 751 were excluded, leaving 11 records whose full text was screened. After evaluation, eight studies were included in the first step of the review (Figure 1). The same screening process was then replicated in the context of a second search performed a few months later, which yielded a total of $n = 1$ article. Details on the second screening process are also reported in Figure 1. In total, $n = 9$ articles were included in this review. Among the included studies, five studies [23–27] were conducted in the United States, two [28,29] in Japan, one in Taiwan [30], and one in Canada [31]. The most recent studies were published in 2021–2022 [23,26,27,31], and two more were published in the years 2017 and 2018 [28,29], while the remaining three were published before 2013 [24,25,30]. Five of the included studies [23,26,27,30,31] refer to slow-onset disasters (i.e., epidemics), while the other four [24,25,28,29] refer to sudden-onset disasters (i.e., earthquake, chemical spill). Two studies [25,30] used interrupted time series analysis, two [23,24] were conducted as a pre-/post-test study, three had a cross-sectional design [26–28], and two had a retrospective cohort or population-based design [29,31]. All studies were aimed at assessing the impact of disasters on ACSC hospitalization rates, except for Wright et al. (2021), which compared health care use and costs of individuals enrolled in Medicaid before the COVID-19 pandemic vs. those enrolled in Medicaid during the first COVID-19 wave. A breakdown of the full characteristics of the included studies is listed in the (Supplementary File S3).

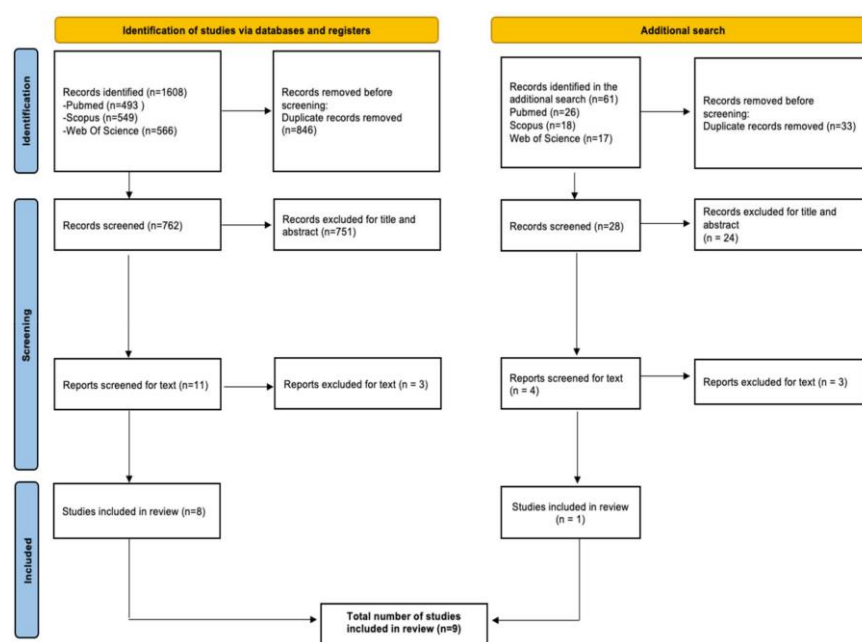


Figure 1. PRISMA checklist for the identification of studies.

3.1. Target Populations

The populations and source data in the included studies were highly heterogeneous. Two studies [24,25] collected data about hospital and emergency department admissions from US Medicaid beneficiaries (medically vulnerable individuals) and used data from Medicaid paid claims. One study [23] focused on non-COVID-19 patients admitted to a non-intensive care unit at a tertiary hospital, using discharge data from electronic health records. Two studies [28,29] analyzed ACSC patients' admissions to the hospital; Sasabuchi et al. (2018) collected data from the National Health Insurance of Kumamoto Prefecture and the Late Elders' Health Insurance, while Sasabuchi et al. (2016) collected data from the Japanese Diagnosis Procedure inpatient database. One study [30] had a population-based sample and relied on inpatient claims of every National Health Insurance beneficiary in Taiwan. Another study [26] collected de-identified insurance claims for adults enrolled in a US-based health maintenance organization network. One study [31] used Canada's National Ambulatory Care Reporting System to obtain population-based data on ACSC hospitalizations. The last study [27] used North Carolina Medicaid claims data and examined different patterns of Medicaid enrollees.

3.2. Types of ACSCs

In total, 18 ACSCs were used in the nine included studies (Supplementary File S4). These can be classified into three groups: (a) chronic ACSCs (i.e., chronic obstructive pulmonary disease (COPD), asthma, congestive heart failure, arterial hypertension (AH), diabetes, angina), (b) acute ACSCs (i.e., dehydration, urinary tract infection, anemia, gastrointestinal bleeding, nutritional deficiencies, seizures, ear–nose–throat infection, cellulitis, pelvic inflammatory disease, dental conditions, gangrene), and (c) vaccine-preventable ACSCs (i.e., influenza, pneumonia). Two studies [28,29] analyzed all three classes independently; three studies [24–26] combined the three classes; one study only considered acute and chronic ACSCs [27]; one study [30] analyzed only chronic ACSCs; and two studies [23,31] analyzed chronic ACSCs, making sure to exclude cases of hospital-acquired pneumonia. For a full list of ACSCs used in each study, see Supplementary File S4.

3.3. Studies' Objectives and Main Results

Five studies examined slow-onset disasters, namely, the 2003 SARS epidemic in Taiwan and the COVID-19 pandemic. Leuchter et al. (2021) compared a 6-month period in 2019 with the same 6-month period in 2020 and found a decrease in hospitalization rates for ACSCs during the COVID-19 pandemic. Huang et al. (2009) performed a population-based interrupted time series analysis to compare actual vs. predicted ACSC hospitalizations during the SARS outbreak, finding lower-than-predicted hospitalization rates for all the selected ACSCs during the SARS outbreak, but increased hospitalization rates for diabetes and hypertension following the period of the outbreak. Becker et al. (2022) compared hospitalization rates before the COVID-19 pandemic (March 2019–February 2020) and during the pandemic period (March 2020–February 2021), finding an overall reduction in all hospitalizations (both non-ACSC and ACSC), with a greater reduction for respiratory-related ACSC hospitalizations. Wright et al. (2021) explored health care use and costs among different types of Medicaid enrollees before and during the COVID-19 pandemic, finding that new Medicaid enrollees during COVID-19 were less likely to be hospitalized for ACSCs than those who enrolled before the COVID-19 pandemic. Rennert-May et al. (2021) compared the most frequent ACSC diagnoses for hospital admissions before and after the implementation of COVID-19 public health measures to determine the impact of COVID-19 on hospital admissions and found that, although hospital admissions did not vary significantly, there was a significant reduction in admissions for chronic respiratory conditions.

The sudden-onset disasters considered in the retrieved studies were the 2005 chlorine spill in Graniteville, South Carolina, the 2011 Great East Japan Earthquake, and the 2016 Kumamoto earthquakes in Japan. Regarding the chlorine spill, Runkle et al. (2012) applied an interrupted time series analysis of 36 months in the pre-disaster phase and 24 months in

the post-disaster phase, finding a decrease in ACSC hospital and emergency department visits. However, a second analysis of the same population, published by the same authors and considering a wider pool of variables, subsequently found a significant increase in post-disaster hospital and emergency department visits, as well as a significant decline in emergency department discharges. Regarding the 2011 Great East Japan Earthquake, Sasabuchi et al. (2016) compared pre-disaster (July 2010–February 2011) with post-disaster ACSC hospitalization rates (July 2012–February 2013) and found a significant increase in acute ACSC admissions, with no increase in admissions for preventable or chronic ACSCs. A retrospective cohort study performed by Sasabuchi et al. (2018) examined ACSC admissions between 15 March and 16 May of each year from 2013 to 2016 to explore the impact of the 2016 Kumamoto earthquakes and identified an increase in admissions within seven days after the earthquakes for vaccine-preventable, acute, and chronic ACSCs, with no significant change 30 days after the earthquakes.

3.4. Vulnerable Populations

Although ACSC hospitalization rates can be used to infer information about demographic and socio-economic factors hampering access to PHC, only Leuchter et al. (2021) and Wright et al. (2021) provided information on disadvantaged groups, namely, those more likely to be hospitalized for ACSCs: African Americans and rural populations.

4. Discussion

The aim of this study was to investigate the extent to which ACSCs have been used in the disaster-related literature, and then to shed light on the potential use of such indicators for the assessment of PHC performance during disasters. The fact that only nine articles matched the inclusion criteria shows that ACSC indicators have rarely been used in disaster research. However, as four out of nine articles were published in the last two years following the global spread of COVID-19, scholarly interest in the topic may be increasing. Scholars should approach the use of the ACSC methodology in disaster research with caution. Following disasters, many factors influence hospitalization for ACSCs, such as disease prevalence, patient health-seeking behavior, the degree of damage to health facilities, and the pre-existing health system organization. Consequently, changes in hospitalization rates for ACSCs and the types of conclusions that can be drawn are highly context-dependent.

Overall, hospitalization rates for ACSCs during disasters were measured to achieve the following main goals: (1) to assess the long-term impact of disasters on public health; (2) to evaluate the performance of the PHC system during disasters; and (3) to identify vulnerable categories of health seekers, namely, those more likely to be hospitalized for ACSCs. Regarding the first goal, studies have shown that patterns of avoidable hospitalizations for ACSCs persisted in the long term after the disaster, explaining the so-called “secondary surge” in health needs that follows in the wake of a disaster. In the works of Runkle et al. (2012, 2013), for example, a sudden-onset disaster caused a secondary surge in PHC needs in the weeks and months after the disaster. Regarding the second goal, studies identified impaired access to PHC services as a possible explanation for the changes in hospitalization rates and, thus, used hospitalization rates for ACSCs as a proxy for PHC functioning. Studies on slow-onset disasters [23,26,27,30,31] reported an overall decrease in ACSC hospitalizations, suggesting better management of health needs at the PHC level during sudden-onset rather than slow-onset disasters. Regarding the third goal, studies examined hospitalizations for ACSCs to identify vulnerable groups, namely, those more likely to be hospitalized for ACSCs. Leuchter [23] and Huang [30] showed that, despite an overall reduction in ACSC hospitalization rates, African Americans and diabetic patients experienced higher rates of hospitalization for chronic ACSCs compared to other populations during the COVID-19 and SARS outbreaks.

Caution should be used when considering hospitalizations for ACSCs as a proxy for PHC functioning during disasters. In fact, many complicating factors influence the relationship between PHC and ACSC hospitalizations following disasters, both at the

patient and health system levels. Therefore, ACSC data must be interpreted alongside other information if they are to yield accurate results about PHC performance [6].

Patients' propensity to seek care in the aftermath of a disaster must be considered. In the event of a disaster, patients are more likely to avoid hospital contact [32]. Those with a chronic disease may only seek care later during flare-ups, which makes it more likely for them to be hospitalized at a later stage [33]. This may decrease the rates of potentially avoidable hospitalizations observed during disasters and alter the relationship between ACSCs and PHC performance. This tendency was observed at the beginning of the COVID-19 pandemic and during the SARS outbreak in 2009 [34–36]. The measurement of ACSC hospitalizations should include longer time periods for additional time series analyses in order to uncover potentially unrecognized underlying trends that may explain the results, and to appropriately factor the propensity for hospitalization into the analysis.

Differences in admission/discharge hospital policies should also be considered. During disasters, there may be changes to such policies or changes in clinicians' conduct that need to be considered since they can alter the interpretation of the ACSC data. As an example, during the COVID-19 pandemic, hospital beds were frequently converted to COVID-19 beds for infected patients, lowering the number of beds available for non-COVID cases [37]. Moreover, physicians might have hesitated to admit or discharge patients amid the pandemic because of a fear of transmission, a lack of possibilities of isolation, or due to a patient's lack of social support. Moreover, patients may be re-hospitalized in nearby hospitals, so re-admissions should also be considered, and hospitals' health information systems should be used to capture this phenomenon and avoid a misinterpretation of data.

The degree of damage to health facilities is another disaster-specific factor that needs to be considered. Information about the degree of damage to hospital facilities (i.e., number of available beds after the disaster vs. standard in-hospital bed capacity) and the degree of disruption to the service of health care facilities needs to be considered to accurately interpret the rates of hospitalization for ACSCs.

The organization of a health system is also relevant when interpreting ACSC data during disasters. When the health system of a country grants access to all patients, regardless of legal entitlements or health conditions, treatment can be administered before health problems become severe, thus reducing rates for hospitalizations [38]. In contrast to what can be generally observed in countries such as the US, where disasters caused by natural hazards usually result in higher rates of hospitalizations for chronic ACSCs [39,40], Japan saw no changes in admissions for chronic ACSCs after the earthquakes in the studies of Sasabuchi et al. (2016, 2018). One possible explanation for this discrepancy may be the fact that, in Japan, PHC centers had absorbed the unmet primary care needs (i.e., chronic disease management) that were present after the earthquake because Japan's health system is based on universal health coverage, as opposed to the insurance-based systems in the US.

To the best of our knowledge, this is the first systematic review addressing the use of ACSCs in disaster research. However, the scant number of studies that met the inclusion criteria demonstrates that the topic is still relatively unexplored. This study was limited by the fact that only publications from languages used by the co-authors and co-researchers were included. However, no potentially relevant article written in any other language (analyzed through the abstracts available in English) could be retrieved. Since this field of inquiry is still in its infancy, each of the reviewed articles used distinct methods and approaches. Therefore, it is still very difficult to generalize about the use of ACSCs in disaster research.

5. Conclusions

In agreement with the H-EDRM framework, it is important to consider the functioning of PHC systems during disasters to plan evidence-based interventions aimed at strengthening PHC disaster preparedness and DM at the PHC level. In the absence of direct methods to assess PHC performance during disasters, the assessment of avoidable hospitalizations can be a reliable strategy to monitor the performance of PHC in times of crisis. Nevertheless,

caution should be used when interpreting changes in disaster-related ACSC hospitalization rates as a proxy for PHC performance, as many factors need to be considered to properly interpret ACSC patterns and increasing/decreasing trends.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijerph19159193/s1>, File S1: Search Queries for PubMed, Web of Science, and Scopus; File S2: Extraction Sheet; File S3: Characteristics of the Studies Included; File S4: ACSCs Used in the Reviewed Articles.

Author Contributions: Conceptualization, A.L.-C. and M.V.; methodology, A.L.-C., M.V. and C.A.; formal analysis, A.L.-C., M.V. and C.A.; writing—original draft preparation, A.L.-C., M.V. and C.A.; writing—review and editing, A.L.-C. and M.V.; supervision, F.B.-A., L.R. and I.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This manuscript is the result of a study conducted in the framework of the International PhD in Global Health, Humanitarian Aid, and Disaster Medicine jointly organized by Università del Piemonte Orientale (UPO) and Vrije Universiteit Brussel (VUB).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bayntun, C. A Health System Approach to All-Hazards Disaster Management: A Systematic Review. *PLoS Curr.* **2012**, *4*, e50081cad5861d. [CrossRef] [PubMed]
2. World Health Organization. *Health Emergency and Disaster Risk Management Framework*; WHO: Geneva, Switzerland, 2019.
3. Redwood-Campbell, L.; Abrahams, J. Primary Health care and Disasters—The Current State of the Literature: What We Know, Gaps and Next Steps. *Prehospital Disaster Med.* **2011**, *26*, 184–191. [CrossRef] [PubMed]
4. Runkle, J.D.; Brock-Martin, A.; Karmaus, W.; Svendsen, E.R. Secondary Surge Capacity: A Framework for Understanding Long-Term Access to Primary Care for Medically Vulnerable Populations in Disaster Recovery. *Am. J. Public Health* **2012**, *102*, e24–e32. [CrossRef] [PubMed]
5. OECD. *Health at a Glance 2015*; OECD Indicators; OECD Publishing: Paris, France, 2015. [CrossRef]
6. Caminal, J.; Starfield, B.; Sánchez, E.; Casanova, C.; Morales, M. The role of primary care in preventing ambulatory care sensitive conditions. *Eur. J. Public Health* **2004**, *14*, 246–251. [CrossRef]
7. Muenchberger, H.; Kendall, E. Predictors of preventable hospitalization in chronic disease: Priorities for change. *J. Public Health Policy* **2010**, *31*, 150–163. [CrossRef]
8. Berlin, C.; Busato, A.; Rosemann, T.; Djalali, S.; Maessen, M. Avoidable hospitalizations in Switzerland: A small area analysis on regional variation, density of physicians, hospital supply and rurality. *BMC Health Serv. Res.* **2014**, *14*, 289. [CrossRef]
9. Billings, J.; Anderson, G.M.; Newman, L.S. Recent Findings on Preventable Hospitalizations. *Health Aff.* **1996**, *15*, 239–249. [CrossRef]
10. Rosano, A.; Loha, C.A.; Falvo, R.; Van Der Zee, J.; Ricciardi, W.; Guasticchi, G.; De Belvis, A.G. The relationship between avoidable hospitalization and accessibility to primary care: A systematic review. *Eur. J. Public Health* **2013**, *23*, 356–360. [CrossRef]
11. World Health Organization Regional Office for Europe. *Assessing Health Services Delivery Performance with Hospitalizations for Ambulatory Care Sensitive Conditions Working Document*; WHO Regional Office for Europe: Copenhagen, Denmark, 2016.
12. Sarmiento, J.; Rocha, J.V.M.; Santana, R. Defining ambulatory care sensitive conditions for adults in Portugal. *BMC Health Serv. Res.* **2020**, *20*, 754. [CrossRef]
13. Hodgson, K.; Deeny, S.R.; Steventon, A. Ambulatory Care-Sensitive Conditions: Their Potential Uses and Limitations. *BMJ Qual. Saf.* **2019**, *28*, 429–433. [CrossRef]
14. Rocha, J.V.M.; Sarmiento, J.; Moita, B.; Marques, A.P.; Santana, R. Comparative research aspects on hospitalizations for ambulatory care sensitive conditions: The case of Brazil and Portugal. *Ciência Saúde Coletiva* **2020**, *25*, 1375–1388. [CrossRef]
15. Helmer, D.A.; Dwibedi, N.; Rowneki, M.; Tseng, C.-L.; Fried, D.; Rose, D.; Jani, N.; Sambamoorthi, U. Mental Health Conditions and Hospitalizations for Ambulatory Care Sensitive Conditions Among Veterans with Diabetes. *Am. Health Drug Benefits* **2020**, *13*, 61.
16. Phillips, K.G.; Wishengrad, J.S.; Houtenville, A.J. Ambulatory Care Sensitive Conditions among All-Payer Claimants with Intellectual and Developmental Disabilities. *Am. J. Intellect. Dev. Disabil.* **2021**, *126*, 203–215. [CrossRef]

17. Dias, B.M.; Zanetti, A.C.B.; Pereira, A.C. Expenses of Hospitalization for Ambulatory Care Sensitive Conditions in the Health Regional Offices of the State of São Paulo. *Einstein* **2021**, *19*, eGS5817. [\[CrossRef\]](#)
18. Fischbach, D. Hospital Costs of Ambulatory Care-Sensitive Conditions in Germany. *Gesundheitswesen* **2016**, *78*, 168–174. [\[CrossRef\]](#)
19. Roos, L.L.; Dragan, R.; Schroth, R.J. Pediatric ambulatory care sensitive conditions: Birth cohorts and the socio-economic gradient. *Can. J. Public Health* **2017**, *108*, e257–e264. [\[CrossRef\]](#)
20. Anderson, T.S.; Karliner, L.S.; Lin, G.A. Association of Primary Language and Hospitalization for Ambulatory Care Sensitive Conditions. *Med. Care* **2019**, *58*, 45–51. [\[CrossRef\]](#)
21. Leventer-Roberts, M.; Cohen-Stavi, C.; Hoshen, M.; Gofer, I.; Sherf, M.; Balicer, R. Analyzing admission rates for multiple ambulatory care-sensitive conditions. *Am. J. Manag. Care* **2020**, *26*, e155–e161. [\[CrossRef\]](#)
22. Walsh, M.E.; Cronin, S.; Boland, F.; Ebell, M.H.; Fahey, T.; Wallace, E. Geographical variation of emergency hospital admissions for ambulatory care sensitive conditions in older adults in Ireland 2012–2016. *BMJ Open* **2021**, *11*, e042779. [\[CrossRef\]](#)
23. Leuchter, R.K.; Villaflores, C.W.A.; Norris, K.C.; Sorensen, A.; Vangala, S.; Sarkisian, C.A. Racial Disparities in Potentially Avoidable Hospitalizations During the COVID-19 Pandemic. *Am. J. Prev. Med.* **2021**, *61*, 235–239. [\[CrossRef\]](#)
24. Runkle, J.R.; Zhang, H.; Karmaus, W.; Brock-Martin, A.; Svendsen, E.R. Long-Term Impact of Environmental Public Health Disaster on Health System Performance. *South. Med. J.* **2013**, *106*, 74–81. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Runkle, J.D.; Zhang, H.; Karmaus, W.; Martin, A.B.; Svendsen, E.R. Prediction of Unmet Primary Care Needs for the Medically Vulnerable Post-Disaster: An Interrupted Time-Series Analysis of Health System Responses. *Int. J. Environ. Res. Public Health* **2012**, *9*, 3384–3397. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Becker, N.V.; Karmakar, M.; Tipirneni, R.; Ayanian, J.Z. Trends in Hospitalizations for Ambulatory Care-Sensitive Conditions During the COVID-19 Pandemic. *JAMA Netw. Open* **2022**, *5*, e222933. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Wright, B.; Anderson, D.; Whitaker, R.; Shrader, P.; Bettger, J.P.; Wong, C.; Shafer, P. Comparing health care use and costs among new Medicaid enrollees before and during the COVID-19 pandemic. *BMC Health Serv. Res.* **2021**, *21*, 1152. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Sasabuchi, Y.; Matsui, H.; Yasunaga, H.; Fushimi, K. Increase in avoidable hospital admissions after the Great East Japan Earthquake. *J. Epidemiol. Community Health* **2017**, *71*, 248–252. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Sasabuchi, Y.; Matsui, H.; Kotani, K.; Lefor, A.K.; Yasunaga, H. Effect of the 2016 Kumamoto earthquakes on preventable hospital admissions: A retrospective cohort study in Japan. *BMJ Open* **2018**, *8*, e021294. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Huang, Y.-T.; Lee, Y.-C.; Hsiao, C.-J. Hospitalization for Ambulatory-care-sensitive Conditions in Taiwan Following the SARS Outbreak: A Population-based Interrupted Time Series Study. *J. Formos. Med. Assoc.* **2009**, *108*, 386–394. [\[CrossRef\]](#)
31. Rennert-May, E.; Leal, J.; Thanh, N.X.; Lang, E.; Dowling, S.; Manns, B.; Wasylak, T.; Ronksley, P.E. The impact of COVID-19 on hospital admissions and emergency department visits: A population-based study. *PLoS ONE* **2021**, *16*, e0252441. [\[CrossRef\]](#)
32. Johnston, S.; Sorel, M.E.; Sidney, S. Effects of the September 11th attacks on urgent and emergent medical evaluations in a Northern California managed care plan. *Am. J. Med.* **2002**, *113*, 556–562. [\[CrossRef\]](#)
33. Saxena, S.; George, J.; Barber, J.; Fitzpatrick, J.; Majeed, A. Association of population and practice factors with potentially avoidable admission rates for chronic diseases in London: Cross sectional analysis. *J. R. Soc. Med.* **2006**, *99*, 81–89. [\[CrossRef\]](#)
34. Wong, L.E.; Hawkins, J.; Langness, S.; Karen, L.; Murrell, P.; Sammann, A. Where Are All the Patients? Addressing Covid-19 Fear to Encourage Sick Patients to Seek Emergency Care. *NEJM Catal.* **2020**, *1*, 1–12. [\[CrossRef\]](#)
35. Lima, C.K.T.; Carvalho, P.M.D.M.; Lima, I.D.A.A.S.; Nunes, J.V.A.D.O.; Saraiva, J.S.; de Souza, R.I.; da Silva, C.G.L.; Neto, M.L.R. The emotional impact of Coronavirus 2019-nCoV (new Coronavirus disease). *Psychiatry Res.* **2020**, *287*, 112915. [\[CrossRef\]](#)
36. Lu, T.-H.; Chou, Y.-J.; Liou, C.-S. Impact of SARS on healthcare utilization by disease categories: Implications for delivery of healthcare services. *Health Policy* **2007**, *83*, 375–381. [\[CrossRef\]](#)
37. McCabe, R.; Schmit, N.; Christen, P.; D'Aeth, J.C.; Løchen, A.; Rizmie, D.; Nayagam, S.; Miraldo, M.; Aylin, P.; Bottle, A.; et al. Adapting hospital capacity to meet changing demands during the COVID-19 pandemic. *BMC Med.* **2020**, *18*, 1–12. [\[CrossRef\]](#)
38. Jones, R. *Oxford Textbook of Primary Medical Care: Clinical Management*; Oxford University Press: Oxford, UK, 2004; Volume 2, pp. 3–9.
39. Fonseca, V.A.; Smith, H.; Kuhadiya, N.; Leger, S.M.; Yau, C.L.; Reynolds, K.; Shi, L.; McDuffie, R.H.; Thethi, T.; John-Kalarickal, J. Impact of a Natural Disaster on Diabetes: Exacerbation of Disparities and Long-Term Consequences. *Diabetes Care* **2009**, *32*, 1632–1638. [\[CrossRef\]](#)
40. Islam, T.; Muntner, P.; Webber, L.S.; Morisky, D.E.; Krousel-Wood, M.A. Cohort Study of Medication Adherence in Older Adults (CoSMO): Extended Effects of Hurricane Katrina on Medication Adherence among Older Adults. *Am. J. Med. Sci.* **2008**, *336*, 105–110. [\[CrossRef\]](#)

Taste and Smell Disorders in COVID-19 Patients at a Local Healthcare Trust in Northern Italy: a Cross-Sectional Study

N. Piu¹, A. Isabella¹, C. Airoidi¹, C. Aleni¹, A. Sarro¹, F. Faggiano^{1,2}

Key words: Covid-19, Loss of smell, Loss of taste, Severity of disease

Parole chiave: Covid-19, Anosmia, Ageusia, Decorso clinico grave

Abstract

Background. In Italy, since the beginning of the COVID-19 pandemic, patients testing positive for SARS-CoV-2 through nasopharyngeal swab have reported taste and smell alterations. As these symptoms are quite uncommon in other respiratory infections, their specificity and prevalence are useful features for the differential diagnosis of COVID-19.

The objective was to describe taste and smell alterations in patients diagnosed with SARS-CoV-2 infection and to define the onset of those disorders during the clinical course of the disease.

Study design. Cross-sectional study.

Methods. This study was conducted on adult patients testing positive for SARS-CoV-2 infection through nasopharyngeal swab at a Local Healthcare Trust in Northern Italy between April 27th and May 27th, 2020. In order to investigate the clinical course, the onset of the first symptoms, smell and/or taste alterations, a phone-based questionnaire was administered during the programming of the second nasopharyngeal swab.

Results. We recruited 168 patients; the mean age was 52 years old, and 94 (56.0%) were female. Among the 135 symptomatic patients, 87 (64.4%) reported taste and smell alterations. Patients with a mild clinical course showed the highest prevalence for taste and smell disorders (76.6%), which were also associated with a more benign clinical course of the disease ($P = 0.0166$). Furthermore, in 33 (37.9%) of these patients, changes in smell and taste perception appeared before the acute phase of the disease. Among the 14 patients with a severe clinical course of SARS-CoV-2 infection, 8 reported a variation of taste and smell perception before the hospitalization, with a median of 4 days (IQR 2-7)

Conclusions. Smell and taste disorders can be listed among the first symptoms of SARS-CoV-2 infection and may anticipate the acute phase of the disease. Noteworthy, they seem to be associated with a more benign clinical course.

¹Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy

²Epidemiological Observatory, Local Healthcare Trust, Vercelli, Italy

Background

In Italy, since the beginning of the COVID-19 pandemic, there have been numerous reports of patients testing positive for SARS-CoV-2 infection through nasopharyngeal swab, recounting taste and smell alterations (1). Moreover, taste and smell alterations, which are not frequently found in other respiratory infections, are among the most frequent and specific symptoms of infection, making their specificity and prevalence particularly useful for the differential diagnosis of COVID-19, especially during the upcoming flu season.

The most frequent symptoms of the disease are fever (83.3%), cough (60.3%), and asthenia (38%), which are often accompanied by chest pain, myalgia, sore throat and dyspnea. Rhinorrhea (3.5%) and nasal congestion (2%), probably due to ageusia and anosmia, are less frequent (2).

Smell and taste alterations (65%) are amongst the earliest and more frequent symptoms of COVID-19 to appear (3). In patients tested positive for SARS-CoV-2, taste and smell alterations are the first symptoms to manifest in 10% and 11% of cases, respectively (4). A study also described their specificity (90.3%) due to estimation of the positive predictive value (5).

The purpose of this study was to describe and ascertain the association between smell and taste disorders and the clinical course of SARS-CoV-2 infection, and to define the temporal association of those symptoms with the acute phase of the disease. In particular, we sought to determine whether they might be associated with a more benign clinical course of the disease.

Methods

Population

Our study population consisted of COVID-19 patients followed by the Local

Healthcare Trust of Vercelli, which consists of all persons residing and healthcare workers (HCWs) in the care homes in this area. Enrolled participants were identified using the current Administrative Health Database (AHDs) of Piedmont Region. For this reason, the HCWs in the care homes, although not resident in this area, were enrolled in the study. On the contrary, the HCWs from the Regional Health Service, whose healing process was followed by the occupational health services of the same Local Healthcare Trust, were not included.

Study design

This was a cross-sectional study conducted between April 27th and May 27th, 2020. A random sample of subjects were included. The phone calls were assigned randomly to four operators by the administrative manager of the contact tracing force working at the Local Healthcare Trust in Vercelli. Only two of them (NP, AI) were involved in the study, and contacted 168 patients out of 351 subjects. At the time of the second nasopharyngeal swab, they were submitted to a phone-based questionnaire.

The survey was structured in order to investigate the clinical course, the time of onset of the first symptoms, smell and/or taste alterations onset, and association of those disorders with the severity of the disease. During this phone interviews, patients were asked whether they had experienced symptoms and when they had begun to exhibit symptoms before diagnosis. On the basis of symptoms and of the severity of the disease, we stratified patients in “asymptomatic”, “mild”, “moderate”, or “severe” according to the WHO severity classification (6). Participants were also asked to evaluate their sense of smell and sense of taste as normal, decreased or lacking. For participants reporting “decreased” or “lacking”, a follow-up question was provided asking how many days before or after the onset of COVID-

Table 1 - Age and sex for different clinical course.

Clinical course	Age	Males	Females	Total
<i>Asymptomatic</i>	52.3±14.4	9(12.2%)	24(25.5%)	33 (19.6%)
Mild	45.9±14.9	19(25.7%)	28 (29.8%)	47 (28.0%)
Moderate	52.5±13.2	27(36.5%)	33(35.1%)	60 (35.7%)
Severe	63.7±9.7	19(25.6%)	9 (9.6%)	28 (16.7%)
Total	52.5±14.5	74	94	168

19 symptoms any sense of smell and taste decreased perception had begun.

Statistical Analysis

Descriptive analysis reporting absolute frequencies and percentages for categorical variables was performed. In addition, mean and standard deviation or median and interquartile range was carried out for numerical ones.

The analysis was conducted jointly on all subjects, but also disjointedly for severity level. To evaluate the association between clinical severity and smell and taste alterations, a chi-square test was performed. In addition, Cochran-Armitage trend test was conducted to take the trend into account.

A logistic model was implemented using, as outcome variable, the alteration of taste and smell, and as covariate the clinical course severity. Odds ratio (OR) and the respective confidence intervals (IC 95%) has been reported. *P*-values lower than 0.05% were considered significant. The analysis was performed using the software STATA (version 14.0).

Results

We enrolled 168 patients. The mean age was 52.5 (SD 14.5) years old, and 94 (56.0%) were female. Of them, 33 (19.6%) were asymptomatic, 47 (28.0%) reported mild symptoms, 60 (35.7%) reported moderate symptoms and 28 (16.7%) had a severe clinical course (oxygen therapy in hospital setting). Demographic characteristics, separated according to disease severity, are described in Table 1.

We found a statistically significant positive association between gender and severity of the disease (*P* = 0.02); males seemed to report a more severe clinical course than females.

Among 135 symptomatic patients, 87 (64.4%) reported taste and smell alterations. Patients with a mild clinical course showed the highest prevalence for taste and smell disorders (76.6%). In addition, an inverse association between the severity of the disease and the presence of smell and taste alterations was found: smell and taste alterations were associated with a more

Table 2 - Distribution of taste/smell alterations in the symptomatic population.

Clinical course	Smell/taste disorder	Total
Mild	36 (76.6%)	47
Moderate	37 (61.7%)	60
Severe	14 (50.0%)	28
Total	87 (64.4%)	135

The *P*-value was computed by Cochran-Armitage (*P* = 0.02)

Table 3 - Taste and smell alteration onset time compared to general symptoms onset.

Clinical course	Before	At the same time	After	Total
Mild	17	12	7	36
Moderate	8	18	11	37
Severe	8	3	3	14
Onset %	37.9%	37.9%	24.1%	100%
Total	33	33	21	87

benign clinical course of the disease ($P = 0.0166$) (Table 2).

In 57.1% ($n=8$) of participants with a severe course of disease and smell and taste disorder, the onset of those symptoms was established before the hospitalization, with a median of 4 days (IQR 2-7) (Table 3). In addition, it is to consider that, for patients who did not show smell and taste alterations before, oxygen-therapy might be the cause itself.

Discussion

The onset of smell and taste alterations (e.g. anosmia, disgeusia, hyposmia, hypogeusia, etc.), considered as one of the most suggestive symptoms related to COVID-19, is used by clinicians as a screening symptom useful to identify suspected cases. Indeed, it has been largely observed and reported a more significant prevalence of anosmia in COVID-19 patients than in patients suffering from different respiratory infections (7).

The primary aim of this cross-sectional study was to ascertain the potential association between smell and taste disorders and the clinical course of SARS-CoV-2 infection, in order to determine whether they might be associated with a more benign clinical course of the disease.

Incidence of these dysfunctions in COVID-19 patients was higher in European countries, including Italy (34 to 86%), North America (19 to 71%) and the Middle East (36 to 98%), lower in Asian populations (8).

Our study confirmed a consistent prevalence (51.7%) of smell and taste disorders in the enrolled participants. Those results are in line with a previous systematic review and meta-analysis that reported a prevalence for olfactory dysfunction of 52.73% (95% CI: 29.64% - 75.23%) among COVID-19 patients, 36.64% (95% CI: 18.31% - 57.24%), using non-validated instruments vs 86.60% (95% CI: 72.95% - 95.95%), using validated instruments (9). Similarly, the prevalence for gustatory dysfunction was 43.9% (95% CI: 20.46% - 68.95%).

Specifically, some cross-sectional studies reported a prevalence of anosmia ranging between 33.9% and 35.7% (21% for patients who have been hospitalized and 64.4%-68% for patients treated at home) (10).

Smell and taste alterations may appear before, concomitantly, or immediately after the onset of the usual symptoms. Based on that, we tried also to better define the temporal onset of those symptoms.

The majority of our study population reported the onset of olfactory and gustatory alterations before the usual symptoms.

In contrast with our results, a study conducted by Lechien et al. found that olfactory dysfunctions appeared mostly after (65.4%) the appearance of general or ENT (ear, nose, throat) symptoms (22.8%) (11). Discrepancies with our results may be due to different methodological approaches, study design, and the fact that we have considered the onset of olfactory and gustatory dysfunction in relation with

symptoms inherent to the ENT, but also with general symptoms.

This cross-sectional study also came with another very interesting finding: a less severe COVID-19 disease is related to a greater prevalence of those dysfunctions.

In an already mentioned systematic review of 18 cohort studies (8), self-reported olfactory and gustatory alteration is considered a favorable prognostic factor in COVID-19 patients, and our results are in line with their ones.

Similarly, another study, not included in the above-mentioned review, reported that loss of smell was also significantly associated with decreased hospitalizations, intensive care unit admissions, intubations, and acute respiratory distress syndrome (12).

This finding may be due to the clinical importance of other symptoms of the disease; patients with severe clinical course, may have not noticed smell or taste alterations.

This work presents some weaknesses that must be taken into account. First, this study was carried out only on Italian population, thus not allowing extension of current finding to non-Italian populations and those results are maybe not completely applicable and generalizable to general populations.

Other limits of the study are represented by the small size of the sub-population with a severe clinical course and the subjective nature of changes in smell and taste perception.

In addition, limitations include the non-utilization of objective olfactory and gustatory testing; therefore, self-reported taste and smell disorders should be validated in future studies with quantitative tests.

Nevertheless, among the strengths of this work, it is possible to cite the use of administrative health data, robust and valid statistical analyses and the “survey approach” that provides information about diagnosis and disease status that deeply mitigates the limitations typical of using claims data (e.g. lack of clinical data).

Conclusions

Our results support previous findings relating to the favorable prognostic nature of taste and smell dysfunctions in COVID-19 patients, providing new insights.

Those symptoms seem to be suggestive in COVID-19 patients and therefore they should be taken into consideration by physicians for COVID-19 clinical diagnosis. Indeed, those alterations act as a worthy biomarker to predict COVID 19 and may anticipate the acute phase of the disease. Although those dysfunctions seem to be associated with a more benign clinical course, further and larger longitudinal studies, with good methodological quality and larger sample size, are warranted in order to confirm this association.

Acknowledgments

Conflict of interest: The authors declare to have no conflicts of interest.

Riassunto

Disturbi del Gusto e dell'Olfatto in pazienti COVID-19 in un'Azienda Sanitaria Locale del Nord Italia: uno studio trasversale

Introduzione. Sin dall'inizio della pandemia di COVID-19 in Italia, i pazienti che risultavano positivi a SARS-CoV-2 tramite tampone nasofaringeo, hanno riportato alterazioni di gusto ed olfatto. Questi sintomi non sono comuni in altre infezioni dell'apparato respiratorio quindi la loro specificità e prevalenza sono utili per la diagnosi differenziale di COVID-19.

L'obiettivo dello studio è stato quello di descrivere ageusia e anosmia in pazienti con infezione da SARS-CoV-2 e identificare l'insorgenza di questi i disordini durante il decorso clinico della malattia.

Disegno dello studio. Studio trasversale.

Metodi. Lo studio è stato condotto su pazienti risultati positivi a SARS-CoV-2 tramite tampone nasofaringeo presso un dipartimento sanitario locale del nord d'Italia, tra il 27/04/2020 e il 27/05/2020. Per indagare il decorso clinico, l'insorgenza dei primi sintomi e la presenza di alterazioni di gusto e olfatto, è stato somministrato un questionario telefonico durante la prenotazione del secondo tampone.

Risultati. Sono stati reclutati 168 pazienti, di questi 94 (56.0%) sono donne e l'età media è di 52 anni. Tra i 135 pazienti sintomatici, 87 (64.4%) hanno riportato alterazioni di gusto e olfatto. Pazienti con una sintomatologia lieve hanno mostrato una prevalenza più alta per alterazioni di gusto e olfatto ($P=0.0166$). Inoltre, in 33 (37.9%) pazienti, le alterazioni di gusto e olfatto sono apparse prima della fase acuta della malattia. Tra i 14 pazienti con decorso clinico grave da SARS-CoV-2, 8 hanno riportato alterazioni di gusto e olfatto prima dell'ospedalizzazione, con una mediana di 4 giorni (IQR 2-7).

Conclusioni. Alterazioni di gusto e olfatto possono essere considerati tra i primi sintomi di infezione da SARS-CoV-2, e potrebbero anticipare la malattia. Inoltre sembrano essere associati con un decorso clinico più benigno.

References

- Ottaviano G, Carecchio M, Scarpa B, Marchese-Ragona R. Olfactory and rhinological evaluations in SARS-CoV-2 patients complaining of olfactory loss 2020. *Rhinology* 2020 Aug 1; **58**(4): 400-1. doi: 10.4193/Rhin20.136. Epub 2020 Apr 27.
- Fu L, Wang B, Yuan T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. *J Infect* 2020 Jun; **80**(6): 656-65. doi: 10.1016/j.jinf.2020.03.041. Epub 2020 Apr 10.
- Grant MC, Geoghegan L, Arbyn M, et al. The prevalence of symptoms in 24,410 adults infected by the novel coronavirus (SARS-CoV-2; COVID-19): A systematic review and meta-analysis of 148 studies from 9 countries. *PLoS One* 2020 June 23; **15**(6): e0234765. doi: 10.1371/journal.pone.0234765.
- Paderno A, Mattavelli D, Rampinelli V, et al. Olfactory and Gustatory Outcomes in COVID-19: A Prospective Evaluation in Nonhospitalized Subjects. *Otolaryngol Head Neck Surg* 2020 Dec; **163**(6): 1144-9. doi: 10.1177/0194599820939538. Epub 2020 Jun 30.
- Salmon D, Bartier S, Hautefort C, et al. Self-reported loss of smell without nasal obstruction to identify COVID-19. The multicenter CORA-NOSMIA cohort study. *J Infect* 2020 Oct; **81**(4): 614-20. doi: 10.1016/j.jinf.2020.07.005. Epub 2020 Jul 7.
- World Health Organization (WHO). Clinical Management of Covid-19. May 27, 2020. Available on: <https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/publications-and-technical-guidance/2020/clinical-management-of-covid-19-interim-guidance,-27-may-2020> [Last accessed: 2021 Mar 16].
- Kanjanaumporn J, Aeumjaturapat S, Snidvongs K, Seresirikachorn K, Chusakul S. Smell and taste dysfunction in patients with SARS-CoV-2 infection: A review of epidemiology, pathogenesis, prognosis, and treatment options. *Asian Pac J Allergy Immunol* 2020 Jun; **38**(2): 69-77. doi: 10.12932/AP-030520-0826.
- Wong DKC, Gendeh HS, Thong HK, et al. A review of smell and taste dysfunction in COVID-19 patients. *Med J Malaysia* 2020 Sep; **75**(5): 574-81.
- Tong JY, Wong A, Zhu D, Fastenberg JH, Tham T. The Prevalence of Olfactory and Gustatory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis. *Otolaryngol Head Neck Surg* 2020 Jul; **163**(1): 3-11. doi: 10.1177/0194599820926473. Epub 2020 May 5.
- Printza A, Constantinidis J. The role of self-reported smell and taste disorders in suspected COVID-19. *Eur Arch Otorhinolaryngol* 2020 Sep; **277**(9): 2625-30. doi: 10.1007/s00405-020-06069-6. Epub 2020 May 23.
- Lechien JR, Chiesa-Estomba CM, De Siati DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020 Aug; **277**(8): 2251-61. doi: 10.1007/s00405-020-05965-1. Epub 2020 Apr 6.
- Foster KJ, Jauregui E, Tajudeen B, Bishehsari F, Mahdavinia M. Smell loss is a prognostic factor for lower severity of coronavirus disease 2019. *Ann Allergy Asthma Immunol* 2020 Oct; **125**(4): 481-3. doi: 10.1016/j.anai.2020.07.023. Epub 2020 Jun 24.

Brief Report

Persistence of Neutralizing Antibodies to SARS-CoV-2 in First Wave Infected Individuals at Ten Months Post-Infection: The UnIRSA Cohort Study

Gloria Griffante ¹, Shikha Chandel ¹ , Daniela Ferrante ¹, Valeria Caneparo ^{1,2} , Daniela Capello ^{1,3}, Valentina Bettio ^{1,3}, Cinzia Borgogna ¹ , Chiara Aleni ¹, Salvatore Esposito ¹, Andrea Sarro ¹, Alessandra Vasile ¹, Marco Comba ¹, Tommaso Testa ¹, Gianmarco Cotrupi ¹, Marco De Andrea ^{2,4} , Sara Bortoluzzi ¹ and Marisa Gariglio ^{1,2,*} 

¹ Department of Translational Medicine, University of Piemonte Orientale, 28100 Novara, Italy; gloria.griffante@uniupo.it (G.G.); shikha.chandel@uniupo.it (S.C.); daniela.ferrante@med.uniupo.it (D.F.); valeria.caneparo@med.uniupo.it (V.C.); daniela.capello@med.uniupo.it (D.C.); valentina.bettio@med.uniupo.it (V.B.); cinzia.borgogna@med.uniupo.it (C.B.); 20032401@studenti.uniupo.it (C.A.); 20017042@studenti.uniupo.it (S.E.); andrea.sarro@uniupo.it (A.S.); 20034878@studenti.uniupo.it (A.V.); marco.comba@uniupo.it (M.C.); tommaso.testa@uniupo.it (T.T.); 20034824@studenti.uniupo.it (G.C.); sara.bortoluzzi@uniupo.it (S.B.)

² Center for Translational Research on Autoimmune and Allergic Disease (CAAD), University of Piemonte Orientale, 28100 Novara, Italy; marco.deandrea@unito.it

³ UPO Biobank, University of Piemonte Orientale, 28100 Novara, Italy

⁴ Department of Public Health and Pediatric Sciences, University of Turin, 10126 Turin, Italy

* Correspondence: marisa.gariglio@med.uniupo.it



Citation: Griffante, G.; Chandel, S.; Ferrante, D.; Caneparo, V.; Capello, D.; Bettio, V.; Borgogna, C.; Aleni, C.; Esposito, S.; Sarro, A.; et al. Persistence of Neutralizing Antibodies to SARS-CoV-2 in First Wave Infected Individuals at Ten Months Post-Infection: The UnIRSA Cohort Study. *Viruses* **2021**, *13*, 2270. <https://doi.org/10.3390/v13112270>

Academic Editor: Caijun Sun

Received: 27 August 2021

Accepted: 8 November 2021

Published: 12 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Longitudinal mapping of antibody-based SARS-CoV-2 immunity is critical for public health control of the pandemic and vaccine development. We performed a longitudinal analysis of the antibody-based immune response in a cohort of 100 COVID-19 individuals who were infected during the first wave of infection in northern Italy. The SARS-CoV-2 humoral response was tested using the COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO Kit (R&D Systems, Bio-Techne, Minneapolis, USA) and pseudotype-based neutralizing antibody assay. Using sequential serum samples collected from 100 COVID-19 recovered individuals from northern Italy—mostly with mild disease—at 2 and 10 months after their first positive PCR test, we show that 93% of them seroconverted at 2 months, with a geometric mean (GeoMean) half-maximal neutralization titer (NT50) of 387.9. Among the 35 unvaccinated subjects retested at 10 months, 7 resulted seronegative, with an 80% drop in seropositivity, while 28 showed decreased anti-receptor binding domain (RBD) and anti-spike (S) IgG titers, with a GeoMean NT50 neutralization titer dropping to 163.5. As an NT50 > 100 is known to confer protection from SARS-CoV-2 re-infection, our data show that the neutralizing activity elicited by the natural infection has lasted for at least 10 months in a large fraction of subjects.

Keywords: SARS-CoV-2; COVID-19; neutralizing humoral response

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with the development of variable levels of antibodies (Abs) with neutralizing activity, which can protect against infection in animal models [1–3]. However, the duration of the serological response in infected subjects and the extent to which such Ab response may be protective against reinfection [4] have still to be fully characterized.

Given the short time SARS-CoV-2 has been studied, information on long-term antibody dynamics is still limited. In this regard, quantitative titer determination of virus-neutralizing Abs (nAbs) is considered an excellent correlate of protection (CoP). Initial

reports pointed to a rapid decline in the humoral response within 3–4 months post-infection [5–7]. More recently, longitudinal studies assessing mid-term kinetics of SARS-CoV-2 infection showed persistent neutralizing antibody responses for up to 8–10 months [8–10], leading to the possibility that nAbs to SARS-CoV-2 may also represent a CoP from emerging SARS-CoV-2 variants of concern.

The aim of this study was to assess the longitudinal profile of anti-spike (S) IgG and anti-recombinant receptor binding domain (RBD) Abs as well as the SARS-CoV-2 neutralizing activity of sera from 100 individuals who were infected during the first wave of SARS-CoV-2 infection in Italy. Although a decline in both IgG levels and neutralizing activity was observed overtime, most of the study subjects still retained a neutralizing activity above the cut-off value (i.e., GeoMean NT50, the reciprocal dilution inhibiting 50% of the infection) of 100, which is considered to be effective in reducing the risk of reinfection [11,12].

2. Material and Methods

2.1. Ethical Statement

Participants were involved and consented under the UPO Biobank study and ethical governance approved by the Ethics Committee of “Maggiore della Carità” Hospital (protocol 427/CE; study No. CE 84/20).

2.2. Human Subjects, Sample, and Data Collection

Blood samples were obtained from individuals enrolled in the UnIRSA (Unveiling the Immune Response against SARS-CoV-2) cohort study. An observational study was carried out on a cohort of 100 individuals with real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR)-confirmed SARS-CoV-2 infection from nasopharyngeal swab that dated from 14 March 2020 to 17 May 2020, thus corresponding to the first wave of infection in Italy. Patient recruitment commenced in May 2020 and continued until June 2020. Recruited patients were followed up until February 2021. The inclusion criteria were (i) one positive rRT-PCR test for SARS-CoV-2 followed by two consecutive negative tests performed 24 h apart, that fulfilled the administrative definition of recovery from COVID-19 as established by the Italian Ministry of Health on 28 February 2020, (ii) age ≥ 18 years, (iii) absence of an immunosuppressive conditions, and (iv) willingness to provide an informed consent. At blood sample collection, an ad-hoc questionnaire was administered to collect data, including socio-demographic characteristics, biometric data (e.g., weight, height, body mass index), and presentation of SARS-CoV-2 infection.

The study participants were stratified according to COVID-19 disease severity as follows: (i) asymptomatic patients, reporting no symptoms, (ii) symptomatic patients, disclosing at least one symptom of those indicated in Table 1 without the need of hospitalization, and (iii) hospitalized patients, requiring hospital assistance due to the severity of COVID-19.

A retrospective chart review was performed on hospitalized participants. Samples were coded and then de-identified prior to analysis. Other efforts to maintain the confidentiality of participants consisted in labeling samples with coded identification numbers. All the data were recorded on the REDCap (<https://www.project-redcap.org/>, accessed on 8 November 2021) web application in compliance with current General Data Protection Regulation (GDPR) and Italian legislation on the protection of sensitive data and privacy.

Table 1. Characteristics of the study population with SARS-CoV-2 infection.

n = 100	
Age at COVID-19 diagnosis (years) median (IQR)	46.5 (33.5, 52.8)
BMI, kg/m ² Median (IQR)	24.2 (21.9, 28.1)
Sex, n (%)	
female	77 (77)
male	23 (23)
Occupation, n (%)	
Physician or paramedical staff	74 (74)
Others	26 (26)
Comorbidities, n (%)	
No comorbidities	53 (53)
allergy	29 (29)
hypertension	14 (14)
autoimmune diseases	13 (3)
asthma	5 (5)
cancer	3 (3)
diabetes	3 (3)
heart disease	2 (2)
Reported Symptoms, n (%)	
No symptoms	16 (16)
asthenia	57 (57)
anosmia	55 (55)
muscle ache	53 (53)
fever	52 (52)
ageusia	49 (49)
headache	47 (47)
cough	44 (44)
diarrhea	35 (35)
runny nose	28 (28)
dyspnea	24 (24)
chest pain	22 (22)
skin manifestations	14 (14)
palpitations	13 (13)
Severity, n (%)	
asymptomatic	16 (16)
symptomatic	74 (74)
hospitalized	10 (10)
Median (IQR) days between positive PCR and first antibody test (M2)	51 (43, 56)
Median (IQR) days between positive PCR and second antibody test (M10)	293 (287, 303)
Vaccinated (among 71 subjects with second antibody test), n (%)	36 (50.7)
One dose, n (%)	21 (29.6)
Two doses, n (%)	15 (21.1)
Median (IQR) days between vaccination (single dose) and second antibody test (M10)	15 (11, 18)
Median (IQR) days between vaccination (second dose) and second antibody test (M10)	8 (3, 10)

2.3. Quantitative Determination of Anti-SARS-CoV-2-Specific Abs

To perform quantitative determination of anti-SARS-CoV-2 Abs, the COVID-SeroIndex, Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO Kit (R&D Systems, Bio-Techne, Minneapolis, MN, USA), which comprises two serial direct enzyme-linked immunosorbent

assays (ELISA), was employed. The immunoassays were used and interpreted following the manufacturer's instructions. Briefly, an initial ELISA was performed to test the reactivity of Abs raised against the recombinant receptor binding domain (RBD) of the SARS-CoV-2 spike protein from the first virus isolate Wuhan-Hu-1 [13,14]. Results were expressed as cut-off index (CI) calculated as the ratio of the corrected sample OD value to the corrected positive control optical density (OD) value. CI values > 0.7 were considered positive. In the quantitative ELISA against the full-length SARS-CoV-2 spike protein (S), the Ab concentration of samples was calculated using a four-parameter logistic (4-PL) curve fit. Values below the limit of quantification (LoQ) of 3.2 AU/mL were considered negative.

2.4. SARS-CoV-2-Specific Neutralizing Antibody Assay

Vero E6 and Vero E6-TMPRSS2—kindly provided by John Hiscott, Pasteur Institute Rome—were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Sigma-Aldrich, Milan, Italy). The replication-competent vesicular stomatitis virus r(VSV)-eGFP-SARS-CoV-2-Δ21 was a kind gift from Sean P.J. Whelan (Washington University School of Medicine, USA) [15]. To grow this virus, Vero E6 cells were infected with a low Multiplicity of Infection (MOI) (0.01) and maintained at 34 °C from then on. Cell supernatants were harvested upon visualization of extensive cytopathic effect and cell detachment at approximately 24 h post-infection (hpi). Upon viral RNA extraction and amplification by RT-PCR, the *spike* gene was sequenced every time the virus was harvested. The virus was titrated by flow cytometry. Serum samples were heat-inactivated at 56 °C for 30 min. Indicated dilutions of sera were incubated with rVSV-SARS-CoV-2-Δ21 at an MOI of 0.05 for 1 h at 37 °C. Ab-virus complexes were added to Vero E6-TMPRSS2 cells in 96-well plates and incubated at 34 °C for 24 h. Subsequently, cells were fixed in 4% formaldehyde (Millipore Sigma, Burlington, MA, USA) containing DAPI for 15 min on ice, when fixative was replaced with PBS. Images were acquired using Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Winooski, VT, USA) in both the DAPI and GFP channels to visualize nuclei and infected cells (i.e., eGFP-positive cells). The raw images (2 × 2 montage) were acquired using 4X objective, processed, and stitched using the default setting.

2.5. Statistical Analysis

Normally distributed data were represented as mean and standard deviation (SD), whereas data following a non-normal distribution were represented as median and interquartile range (IQR). Categorical variables were summarized as counts and percentages. Differences in medians were evaluated using the Mann–Whitney's U test and Wilcoxon Rank signed-rank test for pairwise comparisons. The Kruskal–Wallis test along with the Dunn test for multiple comparisons was used to compare more than two groups. The Bonferroni correction method was applied.

The Spearman's correlation coefficient was used to compute correlation between quantitative variables. A two-sided *p*-value < 0.05 was considered statistically significant. Images and raw data were processed, and the NT50 (the reciprocal dilution inhibiting 50% of the infection) was calculated by plotting and fitting the log of serum dilution versus response to a 4-parameters equation using the Gen5 v3.0 software. Data were processed using Prism software (GraphPad Prism v6.0) and STATA v16 (College Station, TX, USA).

3. Results

3.1. Patient Selection and Magnitude of the Antibody Response to SARS-CoV-2 at Two Months after the First Positive PCR Test (M2)

From 21 May to 19 June 2020, 100 individuals (77 female, 23 male) who were infected by SARS-CoV-2 during the first wave of infection in Italy were enrolled in this study (Table 1). Their median age was 46.5 years (IQR 33.5, 52.8). All study subjects had an RT-PCR-confirmed SARS-CoV-2 infection. While most of these COVID-19 patients (74%)

experienced mild-to-moderate disease or did not report any symptoms (16%), 10% of them required hospitalization without the need of intensive care unit (ICU)-level care.

All patients provided an initial blood sample defined as M2 (approximately two months post-infection), which was taken at a median number of 51 days (interquartile range, IQR 43, 56) from the first positive PCR test. A second longitudinal blood sample, defined as M10 (approximately 10 months post-infection), was taken from 71 COVID-19 patients at a median number of 293 days after the initial sample collection (IQR 287,303 days). During the observation period, the Italian COVID-19 vaccine campaign started and, between 2 January 2021 and 4 February 2021, 21 study subjects received a single dose of mRNA-based SARS-CoV-2 vaccines, whereas 15 obtained two doses, bringing the total number of study subjects with at least one dose to 36 (Figure 1).

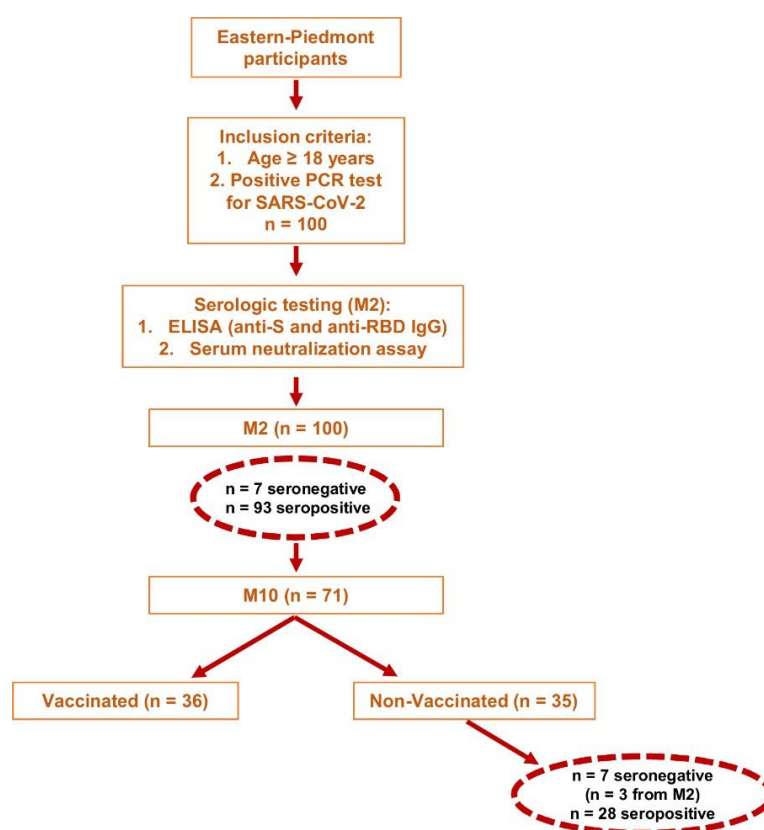


Figure 1. Study design and participants.

Circulating IgG Abs against the SARS-CoV-2 spike antigen were measured using the Kantaro Quantitative SARS-CoV-2 IgG Antibody RUO Kit, which allows detecting IgG against either the RBD or the full-length trimeric spike protein of SARS-CoV-2. At M2, 93% of the subjects showed detectable levels of IgG Abs against both RBD and total S protein, with a positive spearman correlation ($r = 0.9$, $p < 0.0001$) (Figure 2A). In addition, we identified 7 subjects, one of whom had been hospitalized for pneumonia, lacking circulating Abs to SARS-CoV-2 (Table 2). Three of them were retested at M10 but remained seronegative (Figure 1).

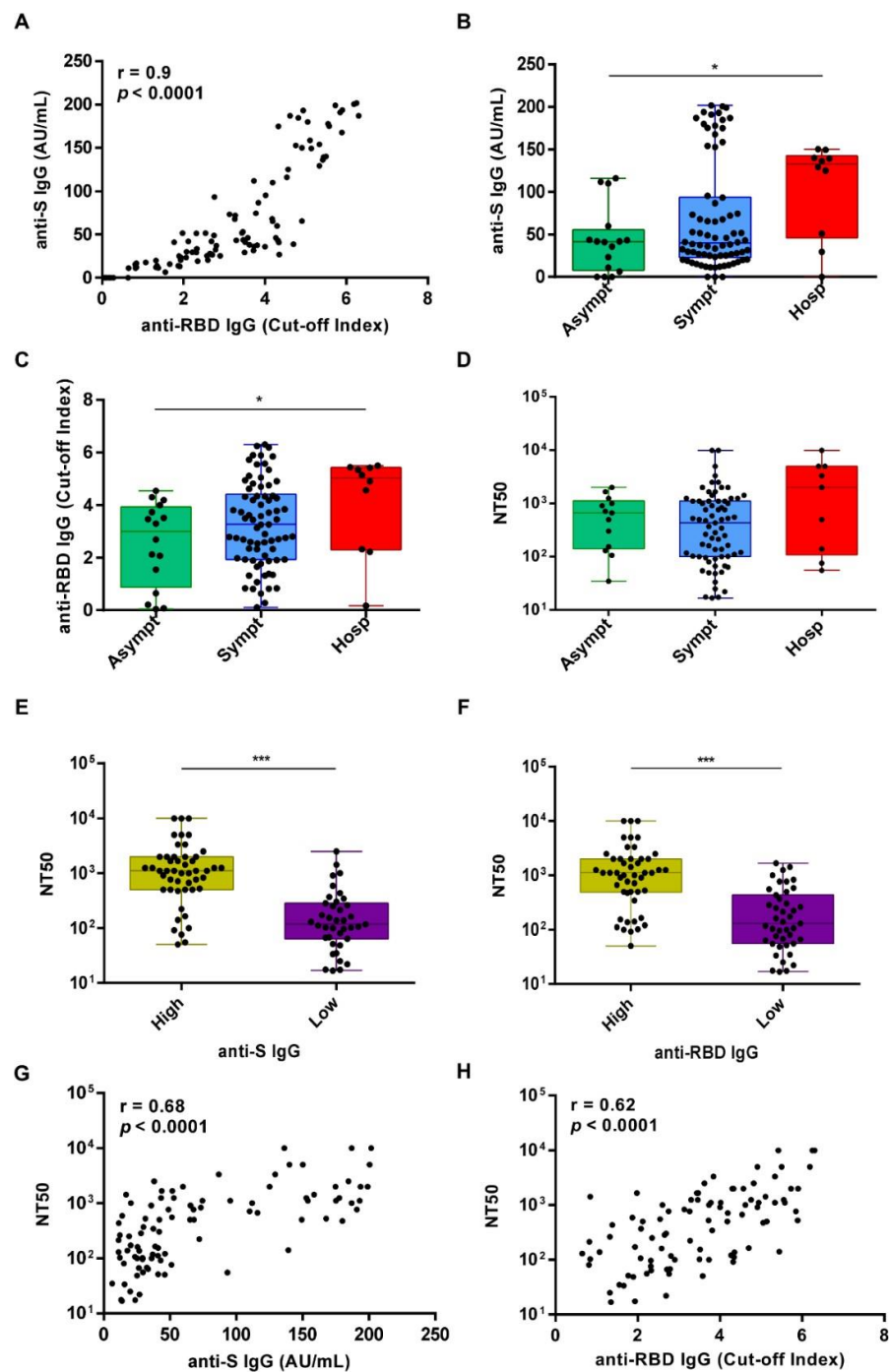


Figure 2. Antibody responses in the study cohort following SARS-CoV-2 infection at 1.5 months (M2) after the first positive PCR test. **(A)** Spearman's correlation between anti-S IgG AU/mL and anti-RBD IgG cut-off index as assessed by ELISA at M2. **(B–D)** The 100 study subjects were categorized into 3 groups according to disease severity (asymptomatic $n = 16$, symptomatic $n = 74$, or hospitalized $n = 10$) and plotted according to anti-S IgG AU/mL **(B)** or anti-RBD IgG cut-off index **(C)** and the neutralization titer expressed as NT50 **(D)**. Solid circles indicate individual values. The p -value among different groups was calculated by Kruskal–Wallis test * $p < 0.05$. **(E)** Box plot distribution of the neutralizing activity expressed as NT50 in the group of high or low anti-S IgG levels—above and below the median, respectively. **(F)** Box plot distribution of the neutralizing activity expressed as NT50 in the group of high or low anti-RBD IgG levels—above and below the median, respectively. In E and F, the solid circles indicate individual values. p -values were determined by two-sided Mann–Whitney test. *** $p < 0.001$. **(G)** Spearman correlation between the neutralization titer expressed as NT50 and the anti-S IgG levels. **(H)** Spearman correlation between the neutralization titer expressed as NT50 and the anti-RBD IgG levels.

Table 2. Clinical and laboratory characteristics of the seronegative individuals at M2.

Subject	Age	Gender	Symptoms	Comorbidities	M2	M10
1	30	F	Asymptomatic	None	55	NA
2	36	M	Symptomatic	None	50	NA
3	63	F	Symptomatic	Cancer	43	NA
4	27	F	Asymptomatic	Allergy	43	NA
5	52	F	Pneumonia	None	43	232
6	58	F	Asymptomatic	None	48	246
7	31	F	Symptomatic	None	27	254

NA, not available.

After patient stratification for COVID-19 disease severity (i.e., asymptomatic, symptomatic, or hospitalized), we observed that both anti-S and anti-RBD Ab levels were significantly higher in the group of hospitalized patients than those detected in asymptomatic cases ($p = 0.04$ and $p = 0.03$, respectively) (Figure 2B,C). A similar trend was observed for the neutralizing titers although not statistically significant ($p = 0.12$) (Figure 2D).

Given that the determination of the neutralizing effects of SARS-CoV-2 spike Abs is critical to understand the protective effects of the immune response, the same sera were also analyzed for their ability to inhibit VSV-SARS-CoV-2- Δ 21 infection of Vero E6 cells, as previously described [15,16]. The latter is a very useful BSL2 surrogate virus whose neutralization profiles strongly correlate with focus-reduction neutralization tests using SARS-CoV-2. We observed that sera with values above the median titer for both anti-S and anti-RBD IgG displayed significantly higher neutralizing activity when compared to those with values below the median value ($p < 0.0001$ for both) (Figure 2E,F), with a positive Spearman correlation $r = 0.68$, $p < 0.0001$ and $r = 0.62$, $p < 0.0001$, respectively (Figure 2G,H). The geometric mean (GeoMean) NT50 neutralization titer was 387.9 (CI 95% 280.8, 535.8) at M2, with 7% of individuals not reaching 50% neutralization at the lowest serum dilution of 1:10.

3.2. Comparative Analysis of the IgG Titers and the Neutralizing Response at Ten Months after the First Positive PCR Test (M10)

Seventy-one subjects provided additional blood samples at M10, which were assessed for the presence of both anti-S and anti-RBD IgG as described for the M2 samples. Notably, at M10, 36 study subjects had received at least one dose of mRNA-based SARS-CoV-2 vaccines.

As the objective of this study was to determine the duration of the immune response to natural infection, assessment of nAbs was only performed for those unvaccinated subjects that had positive ELISA IgG Ab test results ($n = 28/35$). As expected, the IgG titers against S and RBD antigens were both significantly increased in vaccinated subjects at M10 vs. M2 ($p < 0.0001$ for both Abs) (Figure 3A,B, respectively). By contrast, in the 35 unvaccinated individuals the scenario was completely different: 28 of them remained seropositive for both anti-S and anti-RBD Ab, with a positive Spearman correlation $r = 0.8$, $p < 0.0001$ (Figure 3C), while 7 turned out to be seronegative. Three of these latter were already seronegative at M2, whereas the other 4 lost their anti-S and anti-RBD IgG reactivity overtime. Specifically, the percentage of subjects seropositive for both anti-S and anti-RBD IgG within this unvaccinated cohort was 91% (32/35) at M2 vs. 80% (28/35) at M10. When we assessed the levels of the Abs over time, we found that the anti-RBD Abs had significantly decreased between M2 and M10 ($p = 0.0007$), while the anti-S Abs had not significantly changed ($p = 0.38$) (Figure 3A,B, respectively). However, when the subjects were stratified for COVID-19 symptoms, also the decrease in anti-S Abs in symptomatic patients became statistically significant ($p = 0.03$), while it remained unchanged in the other two subgroups (i.e., hospitalized and asymptomatic). A similar trend was also observed for the anti-RBD Ab titers ($p = 0.003$) (Figure 3D,E, respectively).

With regard to the neutralizing Ab titers at M10, we observed that sera displaying values above the median titer for the anti-S IgG displayed significantly higher neutralizing activity compared to that of sera with anti-S values below the median value ($p = 0.0028$) (Figure 4A). When we considered anti-RBD IgG titers below and above the median value,

the trend was similar ($p = 0.0131$) (Figure 4B). The positive Spearman correlation between anti-S or anti-RBD titers and nAb titers was $r = 0.59$ ($p = 0.001$) and $r = 0.51$ ($p = 0.005$), respectively, indicating an overall lower correlation when compared to that observed at M2 (Figure 4C,D, respectively). The GeoMean NT50 neutralization titer was 163.5 (CI 95% 82.1, 325.9) at M10.

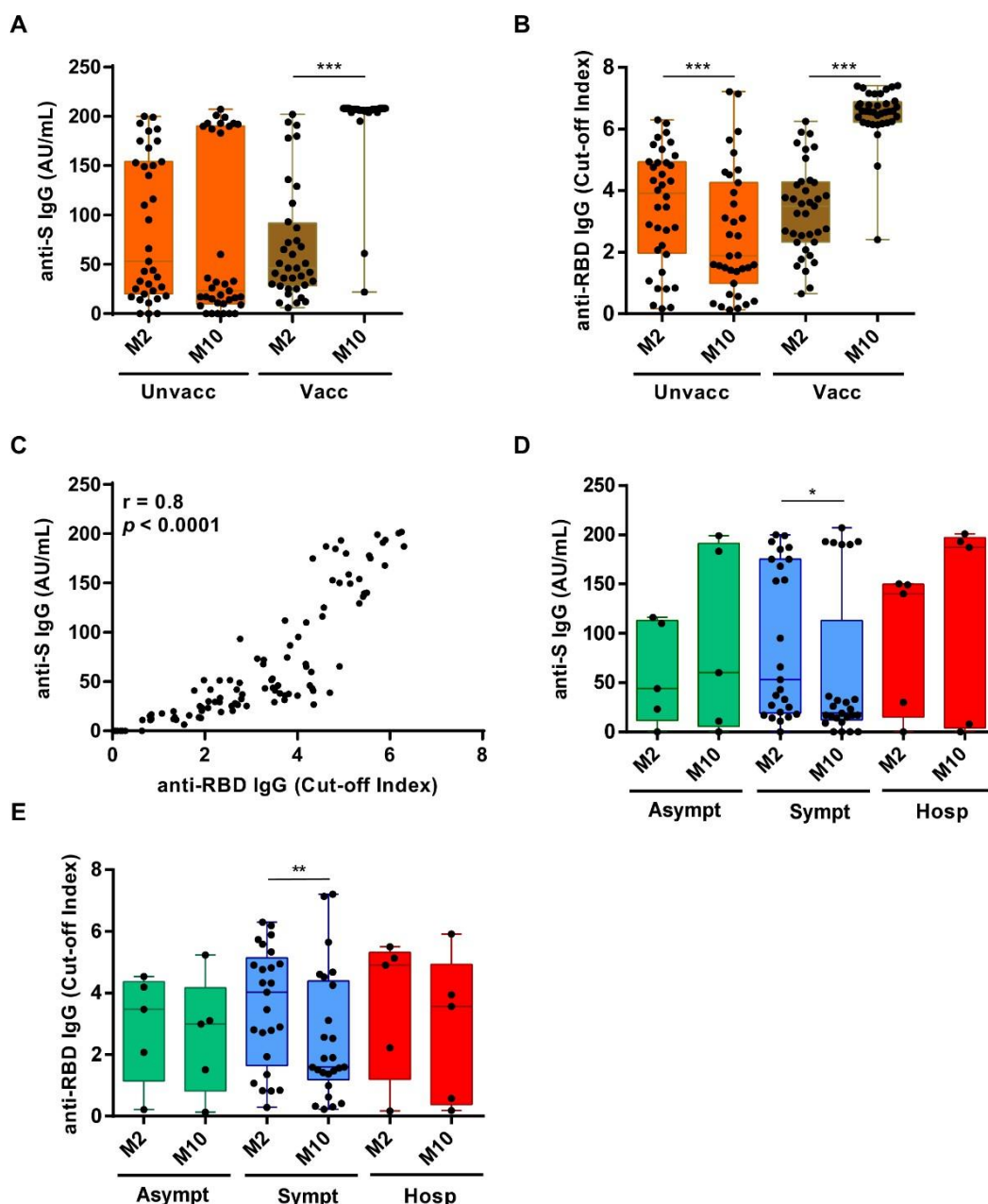


Figure 3. Dynamic changes in anti-S and anti-RBD Ig levels between M2 and M10 after the first PCR-positive test. (A) Box plot distribution of anti-S IgG levels or (B) anti-RBD IgG levels in unvaccinated (left) or vaccinated (right) subjects at M2 vs. M10. Solid circles indicate individual values. p -values were calculated by two-sided Wilcoxon signed-rank tests. $*** p < 0.001$. (C) Spearman correlation between anti-S IgG AU/mL and anti-RBD IgG cut-off index as assessed by ELISA at M10. (D) Comparison of anti-S IgG levels between asymptomatic ($n = 16$), symptomatic ($n = 74$), or hospitalized ($n = 10$) subjects at M2 and M10. p -values were calculated by two-sided Wilcoxon signed-rank tests. $* p < 0.05$. (E) Comparison of anti-RBD cut-off index between asymptomatic $n = 16$, symptomatic $n = 74$, or hospitalized $n = 10$ subjects at M2 or M10. p -values were calculated by two-sided Wilcoxon signed-rank test. $** p < 0.01$.

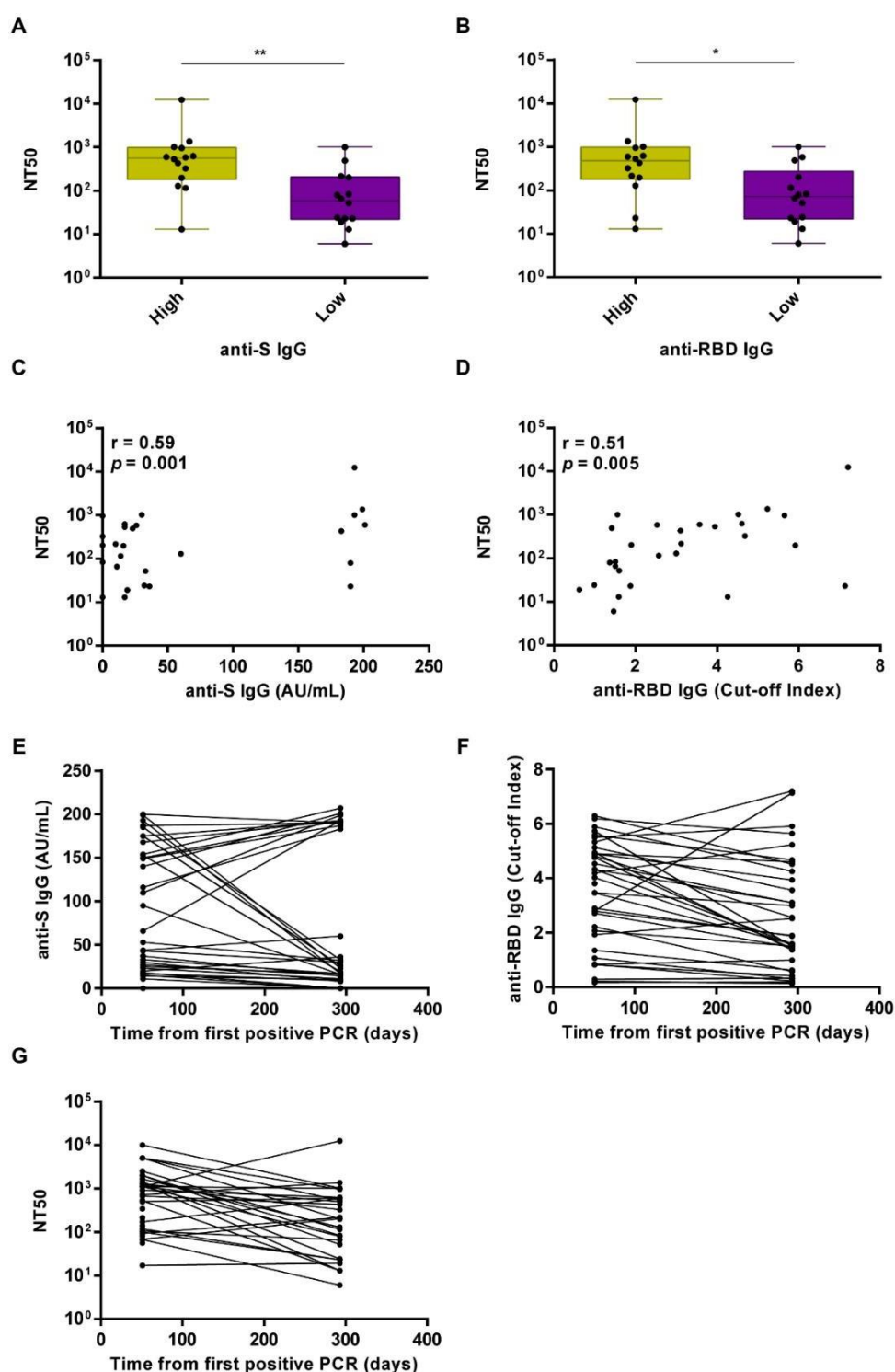


Figure 4. Longitudinal analysis of the neutralizing activity at 10 months (M10) after the first positive PCR test. **(A)** Box plot distribution of the neutralizing activity expressed as NT50 in the group of high or low anti-S IgG levels—above and below the median, respectively. Comparative analysis was performed by Mann-Whitney test. ** $p < 0.01$. **(B)** Box plot distribution of the neutralizing activity expressed as NT50 in the group of high or low anti-RBD IgG levels—above and below the median, respectively. Comparative analysis was performed by Mann-Whitney test. * $p < 0.05$. Solid circles indicate individual values. **(C)** Spearman correlation between anti-S IgG AU/mL and the neutralizing activity expressed as NT50 at M10. **(D)** Spearman correlation between anti-RBD IgG cut-off index and the neutralizing activity expressed as NT50 at M10. **(E)** Longitudinal mapping of anti-RBD IgG cut-off index, anti-S IgG AU/mL **(F)**, or neutralizing activity expressed as NT50 **(G)** in seropositive unvaccinated subjects ($n = 28$) at M2 and M10 after the first PCR-positive test.

We next measured and correlated anti-S, anti-RBD IgG, and nAb levels in the sera of the 28 individuals who had not received the vaccine and remained seropositive at M10. While most of them displayed reduced anti-RBD IgG ($n = 22$) and nAb levels ($n = 21$) at M10 vs. M2, 8 unvaccinated patients still retained nAb titers above the 1:100 cut-off at M10 (48%). On the other hand, 6 subjects showed increased anti-RBD IgG levels at M10 vs. M2, and 6 displayed increased nAb titer values above the 1:100 cut-off at M10 vs. M2, while 1 subject retained low but sustained nAb titer values below the 1:100 cut-off. When we compared anti-S IgG levels at M10 with those at M2, we found them to be reduced in 16 subjects and upregulated in 12 (Figure 4E–G). Interestingly, we found that those who had increased anti-S IgG levels at M10 were older when compared to those who displayed decreased anti-S IgG levels at M10 ($p = 0.01$), as already reported in a series of studies conducted in healthy subjects [17–19]. Next, using the mathematical modeling approach developed by Miles P. Davenport and co-workers, which provides a quantitative prediction of the link between neutralizing antibody levels and clinical protection, we estimated the 50% protective neutralization level against SARS-CoV-2 infection in our cohort to be 78.338 in the M2 group, calculated as 20.2% of the mean level [20]. Using this predictive model and threshold, we found that 80% (28/35) of the subjects in the M2 group and 54.28% (19/35) in the M10 group were above this value. Overall, our findings show that 60% of the subjects in the unvaccinated cohort ($n = 35$) experienced a decline in their serum neutralizing activity at M10, while 20% did show increased nAb levels over time. The antibody levels were below the limit of detection in the remaining 20% of the subjects, indicating that 11.5% completely lost the humoral response, while 8.5% never mounted an immune response.

4. Discussion

In this study, we have performed a longitudinal analysis of the serological responses to SARS-CoV-2 in 100 COVID-19 patients who were infected during the first wave of infection in Italy, of whom 74 were HCWs working at a hospital setting in northern Italy. In these patients, we performed quantitative determination of the anti-RBD and anti-S IgG response to SARS-CoV-2 and evaluated the neutralizing activity of their sera using an in vitro functional assay.

Both anti-RBD and anti-S antibody levels were below the detection limit of the assays in 7% of the subjects after ~50 days from the first SARS-CoV-2 positive PCR test, indicating that these patients did not seroconvert or had already lost their seroconversion at this time point [5,19,21]. At this time point, both anti-RBD and anti-S IgG levels were significantly higher in hospitalized subjects than those observed in asymptomatic or symptomatic patients. Among the 35 subjects who did not receive the vaccine between the M2 and M10 time points, 4 (11%) became seronegative with a drop in seropositivity from 91% at 2 to 80% at 10 months after SARS-CoV-2 infection. Most of the patients displayed lower IgG levels against both S and RBD at M10 vs. M2 (16/35 (42.7%) and 22/35 (62.8%), respectively). Accordingly, the GeoMean NT50 neutralizing titer dropped from 387.9 at M2 to 163.5 at M10. Likewise, the positive Spearman correlation between anti-S or anti-RBD IgG levels and the neutralizing activity of the sera decreased during the interval from M2 to M10, suggesting that the decline in anti-S and RBD IgG levels at longer time points post-infection may not faithfully reflect a similar decline in their neutralizing activity. Recent longitudinal studies aimed at investigating the duration of humoral immune response in COVID-19-recovered individuals reported similar decay kinetics. Overall, the decline appears to occur up to 7–9 months while it is thereafter stabilized at least until 12 months [19,22–24].

SARS-CoV-2 neutralizing Abs are excellent CoP because they can exert their antiviral activity by acting at the mucosal surface, which curbs the initial infection, mostly mediated by secretory IgA whose levels were shown to rise early after natural infection and neutralize the virus even to a greater extent than IgG [25]. In addition, in the bloodstream as circulating IgM or IgG, they block subsequent viral spread and protect from disease progression.

Furthermore, by slowing down the growth rate of SARS-CoV-2, these nAbs may also favor the recruitment of memory B cells capable of neutralizing the infection [26]. Fittingly, vaccine-induced nAbs as well as purified IgG from convalescent animals have been shown to protect non-human primates (NHPs) from infection in a SARS-CoV-2 challenge model [1,2].

One of the major issues in assessing whether nAbs are good proxies for protection from SARS-CoV-2 infection is the determination of a titer cut-off value or range that would allow the identification of those subjects with enough neutralizing activity to make them resistant or less susceptible to reinfection. In spike-based mRNA vaccines studies among people aged 18–55, the GeoMean neutralizing titers after the second dose was 1:181 (day 85), or 1:430 (day 119) depending on the neutralization assay used, while an adenovirus-based vaccine gave an NT50 value of 1:161 or 1:193 (day 42) depending on the neutralization assay used [12]. Another study involving subjects from high attack rate events reported that neutralizing activities in the range 1:100–1:200 were strong enough to prevent infection [11]. Although direct comparisons among the aforementioned studies may suffer from some bias due to the use of different neutralization assays [8,9,15], the emerging concept from this recent body of literature is that an NT50 neutralization titer > 100 is likely to confer protection from SARS-CoV-2 re-infection [11], [reviewed in 12]. Thus, in this context, the fact that we detected a GeoMean NT50 titer of 163.5 at 10 months after the first SARS-CoV-2 positive PCR test would indicate that a significant proportion of individuals (48%) were still within the GeoMean range observed in the vaccinated cohorts. This would suggest that the neutralizing activity elicited by the natural infection may still be effective in a large fraction of subjects (17/35 in our study) after 10 months from the initial infection, even after experiencing a very mild form of COVID-19 disease. More specifically, we found that 74/100 (74%) subjects displayed NT50 neutralizing titer >100 at M2, while in the non-vaccinated subgroup it was 26/35 at M2 (74%) and 17/35 at M10 (48%). In addition, according to the mathematical model developed by Khoury et al., 80% of the subjects at M2 and 54.2% at M10 displayed a neutralization titer that was deemed to be sufficient to provide 50% protection from symptomatic COVID-19 [20].

Our analysis is limited by the reduced sample size, particularly at M10, as half of the study participants received the vaccine in the frame of the Italian vaccination campaign which started during the observational period. However, despite the limited sample size, the availability of two longitudinal measurements, one of which up to 10 months after initial diagnosis, allowed us to achieve adequate statistical power to establish that the neutralizing antibody titer against SARS-CoV-2 were still present in 80% of the study subjects who were infected during the first wave of SARS-CoV-2 infection in Italy. Another limitation of this study is that it is possible that the trends of immune response in recovered, non-vaccinated patients might reflect re-exposure to SARS-CoV-2, particularly in those for whom titers augmented with time. We need also to point out that the requirement for fulfilling the administrative definition of recovery by COVID-19 (as previously described in Material and Methods) provided a homogenizing filter, and thus patients who had lingering detectable SARS-CoV-2 may behave differently. Another limitation is that the role of the memory B cell compartment has not been analyzed in this study. Indeed, the number of RBD-specific memory B cells has been reported to remain stable between 6 and 12 months upon natural infection [19].

Finally, the recombinant VSV expressing SARS-CoV-2 S protein was shown to behave analogously to a clinical isolate of SARS-CoV-2 and to provide comparable results to neutralization tests with the wild-type virus [15]. The r(VSV)-eGFP-based SARS-CoV-2 neutralizing Ab assay may offer utility as a diagnostic tool with which to assess patients' sera neutralizing activity. We postulate it could be predictive of the likelihood of reinfection in the general population.

Author Contributions: Conceptualization: M.G.; Data curation: G.G., S.C.; Formal analysis: D.F.; Funding acquisition: M.G.; Investigation: G.G., S.C., V.C.; Methodology: M.D.A., S.B.; Resources: D.C., V.B., C.A., S.E., A.S., A.V., M.C., T.T., G.C.; Supervision: M.G.; Writing—original draft: G.G., M.G.; Writing—review & editing: C.B., M.D.A., S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the AGING Project, Department of Excellence (DIMET), Università del Piemonte Orientale.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of “Maggiore della Carità” Hospital (protocol 427/CE; study No. CE 84/20) on 15 April 2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank Arabella Fontana and the Novara Health Authority for their support in patient enrollment and database creation. We are also thankful to Carmela Rinaldi and the students from the UPO School of Nursing for their support in blood collection. We are grateful to Sean P.J. Whelan for providing the rVSV-SARS-CoV-2-Δ21. We thank Marcello Arsura for critically reviewing the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mercado, N.B.; Zahn, R.; Wegmann, F.; Loos, C.; Chandrashekar, A.; Yu, J.; Liu, J.; Peter, L.; McMahan, K.; Tostanoski, L.H.; et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* **2020**, *586*, 583–588. [\[CrossRef\]](#)
2. McMahan, K.; Yu, J.; Mercado, N.B.; Loos, C.; Tostanoski, L.H.; Chandrashekar, A.; Liu, J.; Peter, L.; Atyeo, C.; Zhu, A.; et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nat. Cell Biol.* **2021**, *590*, 630–634. [\[CrossRef\]](#)
3. Robbiani, D.F.; Gaebler, C.; Muecksch, F.; Lorenzi, J.C.C.; Wang, Z.; Cho, A.; Agudelo, M.; Barnes, C.O.; Gazumyan, A.; Finkin, S.; et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nat. Cell Biol.* **2020**, *584*, 437–442. [\[CrossRef\]](#)
4. Röltgen, K.; Powell, A.E.; Wirz, O.F.; Stevens, B.A.; Hogan, C.A.; Najeeb, J.; Hunter, M.; Wang, H.; Sahoo, M.K.; Huang, C.; et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci. Immunol.* **2020**, *5*, 956–963. [\[CrossRef\]](#)
5. Marot, S.; Malet, I.; Leducq, V.; Zafilaza, K.; Sterlin, D.; Planas, D.; Gothland, A.; Jary, A.; Dorgham, K.; Bruel, T.; et al. Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. *Nat. Commun.* **2021**, *12*, 844. [\[CrossRef\]](#)
6. Yamayoshi, S.; Yasuhara, A.; Ito, M.; Akasaka, O.; Nakamura, M.; Nakachi, I.; Koga, M.; Mitamura, K.; Yagi, K.; Maeda, K.; et al. Antibody titers against SARS-CoV-2 decline, but do not disappear for several months. *EclinicalMedicine* **2021**, *32*, 100734. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Gaebler, C.; Wang, Z.; Lorenzi, J.C.C.; Muecksch, F.; Finkin, S.; Tokuyama, M.; Cho, A.; Jankovic, M.; Schaefer-Babajew, D.; Oliveira, T.Y.; et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* **2021**, *591*, 639–644. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Dan, J.M.; Mateus, J.; Kato, Y.; Hastie, K.M.; Yu, E.D.; Faliti, C.E.; Grifoni, A.; Ramirez, S.I.; Haupt, S.; Frazier, A.; et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* **2021**, *371*, eabf4063. [\[CrossRef\]](#)
9. Pradenas, E.; Trinité, B.; Urrea, V.; Marfil, S.; Ávila-Nieto, C.; de la Concepción, M.L.R.; Tarrés-Freixas, F.; Pérez-Yanes, S.; Roviro, C.; Ainsua-Enrich, E.; et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. *Med* **2021**, *2*, 313–320.e4. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Levi, R.; Ubaldi, L.; Pozzi, C.; Angelotti, G.; Sandri, M.T.; Azzolini, E.; Salvatici, M.; Savevski, V.; Mantovani, A.; Rescigno, M. The antibody response to SARS-CoV-2 infection persists over at least 8 months in symptomatic patients. *Commun. Med.* **2021**, *1*, 1–9. [\[CrossRef\]](#)
11. Addetia, A.; Crawford, K.H.D.; Dingens, A.; Zhu, H.; Roychoudhury, P.; Huang, M.-L.; Jerome, K.R.; Bloom, J.D.; Greninger, A.L. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J. Clin. Microbiol.* **2020**, *58*, e02107–20. [\[CrossRef\]](#)
12. Koch, T.; Mellinghoff, S.; Shamsrizi, P.; Addo, M.; Dahlke, C. Correlates of Vaccine-Induced protection against SARS-CoV-2. *Vaccines* **2021**, *9*, 238. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 265–269. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Amanat, F.; Stadlbauer, D.; Strohmeier, S.; Nguyen, T.H.O.; Chromikova, V.; McMahon, M.; Jiang, K.; Arunkumar, G.A.; Jurczyszak, D.; Polanco, J.; et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat. Med.* **2020**, *26*, 1033–1036. [\[CrossRef\]](#) [\[PubMed\]](#)

15. Case, J.B.; Rothlauf, P.W.; Chen, R.E.; Liu, Z.; Zhao, H.; Kim, A.S.; Bloyet, L.-M.; Zeng, Q.; Tahan, S.; Droit, L.; et al. Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2. *Cell Host Microbe* **2020**, *28*, 475–485.e5. [[CrossRef](#)] [[PubMed](#)]
16. Borgogna, C.; De Andrea, M.; Griffante, G.; Lai, A.; Bergna, A.; Galli, M.; Zehender, G.; Castello, L.; Ravanini, P.; Cattrini, C.; et al. SARS-CoV-2 reinfection in a cancer patient with a defective neutralizing humoral response. *J. Med. Virol.* **2021**, *93*, 6444–6446. [[CrossRef](#)]
17. Vanshylla, K.; Di Cristanziano, V.; Kleipass, F.; Dewald, F.; Schommers, P.; Gieselmann, L.; Gruell, H.; Schlotz, M.; Ercanoglu, M.S.; Stumpf, R.; et al. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. *Cell Host Microbe* **2021**, *29*, 917–929.e4. [[CrossRef](#)]
18. Glück, V.; Grobecker, S.; Tydykov, L.; Salzberger, B.; Glück, T.; Weidlich, T.; Bertok, M.; Gottwald, C.; Wenzel, J.J.; Gessner, A.; et al. SARS-CoV-2-directed antibodies persist for more than six months in a cohort with mild to moderate COVID-19. *Infection* **2021**, *49*, 739–746. [[CrossRef](#)]
19. Li, C.; Yu, D.; Wu, X.; Liang, H.; Zhou, Z.; Xie, Y.; Li, T.; Wu, J.; Lu, F.; Feng, L.; et al. Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in Wuhan. *Nat. Commun.* **2021**, *12*, 1–9. [[CrossRef](#)]
20. Khoury, D.S.; Cromer, D.; Reynaldi, A.; Schlub, T.E.; Wheatley, A.K.; Juno, J.A.; Subbarao, K.; Kent, S.J.; Triccas, J.A.; Davenport, M.P. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **2021**, *27*, 1205–1211. [[CrossRef](#)]
21. Wajnberg, A.; Amanat, F.; Firpo, A.; Altman, D.R.; Bailey, M.J.; Mansour, M.; McMahon, M.; Meade, P.; Mendu, D.R.; Muellers, K.; et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* **2020**, *370*, 1227–1230. [[CrossRef](#)] [[PubMed](#)]
22. Vacharathit, V.; Srichatrapimuk, S.; Manopwisedjaroen, S.; Kirdlarp, S.; Srisaowakarn, C.; Setthaudom, C.; Inrueangsri, N.; Pisitkun, P.; Kunakorn, M.; Hongeng, S.; et al. SARS-CoV-2 neutralizing antibodies decline after one year and patients with severe COVID-19 pneumonia display a unique cytokine profile. *Int. J. Infect. Dis.* **2021**, *112*, 227–234. [[CrossRef](#)]
23. Petersen, M.S.; Hansen, C.B.; Kristiansen, M.F.; Fjallabak, J.P.; Larsen, S.; Hansen, J.L.; Jarlhelt, I.; Pérez-Alós, L.; Steig, B.Á.; Christiansen, D.H.; et al. SARS-CoV-2 natural antibody response persists for at least 12 months in a nationwide study from the Faroe Islands. *Open Forum Infect. Dis.* **2021**, *8*, ofab378. [[CrossRef](#)] [[PubMed](#)]
24. Wang, Z.; Muecksch, F.; Schaefer-Babajew, D.; Finklin, S.; Viant, C.; Gaebler, C.; Hoffmann, H.-H.; Barnes, C.O.; Cipolla, M.; Ramos, V.; et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nat. Cell Biol.* **2021**, *595*, 426–431. [[CrossRef](#)] [[PubMed](#)]
25. Sterlin, D.; Mathian, A.; Miyara, M.; Mohr, A.; Anna, F.; Claër, L.; Quentric, P.; Fadlallah, J.; Devilliers, H.; Ghillani, P.; et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci. Transl. Med.* **2021**, *13*. [[CrossRef](#)]
26. Corti, D.; Lanzavecchia, A. Broadly Neutralizing Antiviral Antibodies. *Annu. Rev. Immunol.* **2013**, *31*, 705–742. [[CrossRef](#)] [[PubMed](#)]

Acknowledgments

“At times, our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us”

These acknowledgements are lengthy, as I was lucky to have met such smart, enlightening, and supportive professors, colleagues, and friends over the past three years.

My first and biggest thank you goes to my supervisor, Professor Fabrizio Faggiano. I will be forever grateful for all the possibilities, opportunities, and support you have given me. Your words of motivation have been fundamental. Thank you for encouraging me to step out into the “world.” Without I would not have achieved what I have today.

Another huge thank you goes to my co-supervisor, Professor Saverio Stranges. Thank you for bringing me to Canada and Western University. This experience has changed my life, both personally and professionally. Thank you for being a source of inspiration and an incredible mentor, especially in the hardest moments of my career. You have always taken the time to listen to me, even in the busiest moments.

I also want to thank Professor Daniela Capello and my colleagues at UPO. The years we have worked together have been important to my professional and personal growth. I want to thank my colleagues, in no order, who have supported (and listened to) me during my time at UPO.

I was lucky enough to meet people who made my time in Canada incredible. I want to thank the professors and colleagues I met at Western University, particularly Dr. Piotr Wilk, who has always taken the time to provide guidance, share insights, and support my research (sometimes attempts).

Thank you to all the Italians there (as I would call them, the Italian Team of London, Ontario) who made me feel so at home when home was so far away.

Thank you to Marta for showing me Canadian life and how fun it was. Thank you for bringing me into your life and showing me incredible support. Thank you for the long talks on Bowman Street; I am forever grateful.

I must take a few lines to thank Virginia, who has been with me since my Canadian day one and has never gone away. Virginia, you are a source of admiration and strength. You are one of the people I admire the most. I am sure I would not have made it this far without your words and encouragement. Thank you for always being there; I know that I can count on you. Thank you for being my supporter and always lifting me up. I am so lucky to be able to call you my dearest friend.

Thank you to the people I met at Maastricht University (UM) and to all the professors I met there, particularly my supervisor, Dr. Gonnie Klabbers. Each of you, in your very different ways, has deeply changed my perspective of what health is and how it is intertwined in every aspect of life. Thank you for teaching me what it means to “listen to the silences,” to pay attention to what is not said, to the voices often ignored. I have promised to take this teaching with me into everything I will ever do.

Sometimes, you are fortunate enough that your colleagues become some of the closest people in your life, and I was lucky to experience this. I met incredible, talented, and passionate individuals who quickly became my points of reference. Each of them has shown me unwavering support and unconditional love. Thank you, Paula, Begum, Kate, Mariona, Lisa, Wiesje, and Valentina, for showing me that friendships can thrive across countries and for celebrating every accomplishment together. I have nothing but love and gratitude for each of you.

Thank you to my parents, to whom this thesis is dedicated. Thank you for giving me your all, even when it meant letting me leave across the world (or now, a bit closer). I can only imagine how hard it must be. Thank you for allowing me to follow my dreams. Thank you for loving me unconditionally. Everything I am and have accomplished is you, and I hope to always make you proud.

Thank you to my family for being nothing but supportive. I am incredibly lucky. Thank you to my little cousins, Fabrizia and Flavia; my biggest wish for you is to be happy and free to chase whatever dream you have in this life.

Thank you to my friends Stefano, Marco, and Luca for being there every step of this very long journey; I appreciate it more than I can show.

Even if not formally during my doctorate, I must thank the friends I met in Brussels during the Bluebook traineeship at HaDEA. What a rollercoaster!! I have loved every single day of the past five months. Thank you for being with me through every (and it is meant literally) hard workday, networking event, karaoke night and exam simulation. Thank you, Margherita and Cecilia. We still have a lot to do together.

Finally, thank you to everyone who has been part of this journey, whether mentioned here or not. Your support, kindness, and belief in me have carried me through the most challenging and rewarding moments of my life.

This thesis is not just my achievement; it reflects the effort, encouragement, and inspiration of all the incredible people I have been privileged to know. As I move forward, I carry with me your lessons, love, and belief that anything is possible with perseverance and the right people by your side. Thank you for making this journey unforgettable

References

- Ahmed, S., & Shahid, R. (2012). Disparity in cancer care: A Canadian perspective. *Current Oncology*, 19(6), 376–382. <https://doi.org/10.3747/co.19.1177>
- Ahmed, S., Shommu, N. S., Rumana, N., Barron, G. R. S., Wicklum, S., & Turin, T. C. (2015). Barriers to Access of Primary Healthcare by Immigrant Populations in Canada: A Literature Review. *Journal of Immigrant and Minority Health*, 18(6), 1522–1540. <https://doi.org/10.1007/s10903-015-0276-z>
- Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology*, 56(6), 893–897. <https://doi.org/10.1037/0022-006x.56.6.893>
- Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. F. (1996). Comparison of Beck Depression Inventories-IA and-II in Psychiatric outpatients. *Journal of Personality Assessment*, 67(3), 588–597. https://doi.org/10.1207/s15327752jpa6703_13
- Bloom, D. E., Canning, D., & Lubet, A. (2015). Global Population Aging: Facts, Challenges, Solutions & Perspectives. *Daedalus*, 144(2), 80–92. https://doi.org/10.1162/daed_a_00332
- Brabete, A. C. (2017). Examining migrants' health from a gender perspective. In *Elsevier eBooks* (pp. 231–250). <https://doi.org/10.1016/b978-0-12-803864-2.00008-0>
- Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA a Cancer Journal for Clinicians*, 74(3), 229–263. <https://doi.org/10.3322/caac.21834>

Brayne, C., & Moffitt, T. E. (2022). The limitations of large-scale volunteer databases to address inequalities and global challenges in health and aging. *Nature Aging*, 2(9), 775–783. <https://doi.org/10.1038/s43587-022-00277-x>

Bretthauer, M., Wieszczy, P., Løberg, M., Kaminski, M. F., Werner, T. F., Helsingen, L. M., Mori, Y., Holme, Ø., Adami, H., & Kalager, M. (2023). Estimated lifetime gained with cancer screening tests. *JAMA Internal Medicine*, 183(11), 1196. <https://doi.org/10.1001/jamainternmed.2023.3798>

Buyse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research*, 28(2), 193–213. [https://doi.org/10.1016/0165-1781\(89\)90047-4](https://doi.org/10.1016/0165-1781(89)90047-4)

Cancer Care Ontario. (2020). Ontario Cancer Screening Performance Report. In <https://www.cancercareontario.ca/en/cancer-care-ontario/programs/screening-programs/screening-performance-report-2020>.

Cancer Care Ontario. (2021). *Breast Cancer Screening*. <https://www.cancercareontario.ca/en/types-of-cancer/breast-cancer/screening>

Chen, C., Lim, J., Koh, J., Beard, J., Rowe, J. W., Rowe, J., Antonucci, T., Berkman, L., Börsch-Supan, A., Carstensen, L., Goldman, D., Fried, L., Furstenberg, F., Jackson, J., Kohli, M., Olshansky, J., Rehkopf, D., Rother, J., & Zissimopoulos, J. (2024). A global analysis of adaptation to societal aging across low-, middle- and high-income countries using the Global Aging Society Index. *Nature Aging*. <https://doi.org/10.1038/s43587-024-00772-3>

Darin-Mattsson, A., Fors, S., & Kåreholt, I. (2017). Different indicators of socioeconomic status and their relative importance as determinants of health in old age. *International Journal for Equity in Health*, 16(1). <https://doi.org/10.1186/s12939-017-0670-3>

Dey, S. (2014). Preventing breast cancer in LMICs via screening and/or early detection: The real and the surreal. *World Journal of Clinical Oncology*, 5(3), 509.

<https://doi.org/10.5306/wjco.v5.i3.509>

Ding, L., Wang, J., Greuter, M. J. W., Goossens, M., Van Hal, G., & De Bock, G. H. (2022). Determinants of Non-Participation in Population-Based Breast Cancer Screening: A Systematic Review and Meta-Analysis. *Frontiers in Oncology*, 12.

<https://doi.org/10.3389/fonc.2022.817222>

Durand-Moreau, Q., Lafontaine, J., & Ward, J. (2022). Work and health challenges of Indigenous people in Canada. *The Lancet Global Health*, 10(8), e1189–e1197.

[https://doi.org/10.1016/s2214-109x\(22\)00203-0](https://doi.org/10.1016/s2214-109x(22)00203-0)

Eby, P. R., Ghate, S., & Hooley, R. (2022). The Benefits of Early Detection: Evidence from modern International Mammography Service screening programs. *Journal of Breast Imaging*, 4(4), 346–356. <https://doi.org/10.1093/jbi/wbac041>

Emery, J. D., Shaw, K., Williams, B., Mazza, D., Fallon-Ferguson, J., Varlow, M., & Trevena, L. J. (2013). The role of primary care in early detection and follow-up of cancer. *Nature Reviews Clinical Oncology*, 11(1), 38–48. <https://doi.org/10.1038/nrclinonc.2013.212>

European Commission. (2021). *Europe's Beating Cancer Plan*.

https://health.ec.europa.eu/document/download/26fc415a-1f28-4f5b-9bfa-54ea8bc32a3a_en

European Commission. (2022). *Communication from the Commission to the European Parliament and the Council: Strengthening Prevention through Early Detection – A New EU Approach on Cancer Screening* (COM(2022) 474 final).

- Exarchakou, A., Kipourou, D., Belot, A., & Rachet, B. (2022). Socio-economic inequalities in cancer survival: how do they translate into Number of Life-Years Lost? *British Journal of Cancer*, 126(10), 1490–1498. <https://doi.org/10.1038/s41416-022-01720-x>
- Feeney, J. A., & Ryan, S. M. (1994). Attachment style and affect regulation: Relationships with health behavior and family experiences of illness in a student sample. *Health Psychology*, 13(4), 334–345. <https://doi.org/10.1037/0278-6133.13.4.334>
- Ferdous, M., Goopy, S., Yang, H., Rumana, N., Abedin, T., & Turin, T. C. (2019). Barriers to breast cancer screening among immigrant populations in Canada. *Journal of Immigrant and Minority Health*, 22(2), 410–420. <https://doi.org/10.1007/s10903-019-00916-3>
- Ferdous, M., Lee, S., Goopy, S., Yang, H., Rumana, N., Abedin, T., & Turin, T. C. (2018). Barriers to cervical cancer screening faced by immigrant women in Canada: a systematic scoping review. *BMC Women S Health*, 18(1). <https://doi.org/10.1186/s12905-018-0654-5>
- Fuentes, J. D. B., Morgan, E., De Luna Aguilar, A., Mafra, A., Shah, R., Giusti, F., Vignat, J., Znaor, A., Musetti, C., Yip, C., Van Eycken, L., Jedy-Agba, E., Piñeros, M., & Soerjomataram, I. (2023). Global stage distribution of breast Cancer at diagnosis. *JAMA Oncology*, 10(1), 71. <https://doi.org/10.1001/jamaoncol.2023.4837>
- Gilbert, F. J., & Pinker-Domenig, K. (2019). Diagnosis and staging of breast cancer: when and how to use mammography, tomosynthesis, ultrasound, Contrast-Enhanced Mammography, and magnetic resonance imaging. In *IDKD Springer series* (pp. 155–166). https://doi.org/10.1007/978-3-030-11149-6_13
- Glymour, M. M., Avendano, M., & Kawachi, I. (2014). Socioeconomic Status and Health. In *Oxford University Press eBooks* (pp. 17–62). <https://doi.org/10.1093/med/9780195377903.003.0002>

- Gold, N., Christensen, R. a. G., Arneja, J., Aminoleslami, A., Anderson, G. M., & Brooks, J. D. (2023). Screening behaviours, demographics, and stage at diagnosis in the publicly funded Ontario Breast Screening Program. *Breast Cancer Research and Treatment*, 198(3), 523–533. <https://doi.org/10.1007/s10549-022-06848-1>
- Gøtzsche, P. C., & Jørgensen, K. J. (2013). Screening for breast cancer with mammography. *Cochrane Library*, 2013(6). <https://doi.org/10.1002/14651858.cd001877.pub5>
- Gunsoy, N. B., Garcia-Closas, M., & Moss, S. M. (2012). Modelling the overdiagnosis of breast cancer due to mammography screening in women aged 40 to 49 in the United Kingdom. *Breast Cancer Research*, 14(6). <https://doi.org/10.1186/bcr3365>
- Hagströmer, M., Oja, P., & Sjöström, M. (2006). The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutrition*, 9(6), 755–762. <https://doi.org/10.1079/phn2005898>
- Hanske, J., Meyer, C. P., Sammon, J. D., Choueiri, T. K., Menon, M., Lipsitz, S. R., Noldus, J., Nguyen, P. L., Sun, M., & Trinh, Q. (2016). The influence of marital status on the use of breast, cervical, and colorectal cancer screening. *Preventive Medicine*, 89, 140–145. <https://doi.org/10.1016/j.ypmed.2016.05.017>
- Heer, E., Harper, A., Escandor, N., Sung, H., McCormack, V., & Fidler-Benaoudia, M. M. (2020). Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. *The Lancet Global Health*, 8(8), e1027–e1037. [https://doi.org/10.1016/s2214-109x\(20\)30215-1](https://doi.org/10.1016/s2214-109x(20)30215-1)
- Henderson, J. T., Webber, E. M., Weyrich, M., Miller, M., & Melnikow, J. (2024, April 1). *Introduction*. Screening for Breast Cancer: A Comparative Effectiveness Review for the U.S.

Preventive Services Task Force - NCBI Bookshelf.

<https://www.ncbi.nlm.nih.gov/books/NBK603793/#ch1.s1>

Hsu, Y., & Lo, H. (2019). The Impacts of Population Aging on Saving, Capital Formation, and Economic Growth. *American Journal of Industrial and Business Management*, 09(12), 2231–2249. <https://doi.org/10.4236/ajibm.2019.912148>

ISS. (2014). *Sorveglianza Passi d'Argento*. EpiCentro. <https://www.epicentro.iss.it/passi-argento/info/info>

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *SLEEP*, 14(6), 540–545. <https://doi.org/10.1093/sleep/14.6.540>

Jordan, S., Watkins, A., Storey, M., Allen, S. J., Brooks, C. J., Garaiova, I., Heaven, M. L., Jones, R., Plummer, S. F., Russell, I. T., Thornton, C. A., & Morgan, G. (2013). Volunteer bias in recruitment, retention, and blood sample donation in a randomised controlled trial involving mothers and their children at six months and two years: a longitudinal analysis. *PLoS ONE*, 8(7), e67912. <https://doi.org/10.1371/journal.pone.0067912>

Kammin, E. J. (2022). The 6-Minute Walk Test: Indications and guidelines for use in outpatient practices. *The Journal for Nurse Practitioners*, 18(6), 608–610. <https://doi.org/10.1016/j.nurpra.2022.04.013>

Katalinic, A., Eisemann, N., Kraywinkel, K., Noftz, M. R., & Hübner, J. (2019). Breast cancer incidence and mortality before and after implementation of the German mammography screening program. *International Journal of Cancer*, 147(3), 709–718. <https://doi.org/10.1002/ijc.32767>

Khan, H. T. A., Addo, K. M., & Findlay, H. (2024). Public Health Challenges and Responses to the Growing Ageing Populations. *Public Health Challenges*, 3(3).

<https://doi.org/10.1002/puh2.213>

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Benbrahim-Tallaa, L., Bouvard, V., Bianchini, F., & Straif, K. (2015a). Breast-Cancer Screening — Viewpoint of the IARC Working Group. *New England Journal of Medicine*, 372(24), 2353–2358.

<https://doi.org/10.1056/nejmsr1504363>

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Benbrahim-Tallaa, L., Bouvard, V., Bianchini, F., & Straif, K. (2015b). Breast-Cancer Screening — Viewpoint of the IARC Working Group. *New England Journal of Medicine*, 372(24), 2353–2358.

<https://doi.org/10.1056/nejmsr1504363>

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Benbrahim-Tallaa, L., Bouvard, V., Bianchini, F., & Straif, K. (2015c). Breast-Cancer Screening — Viewpoint of the IARC Working Group. *New England Journal of Medicine*, 372(24), 2353–2358.

<https://doi.org/10.1056/nejmsr1504363>

Li, S., He, Y., Liu, J., Chen, K., Yang, Y., Tao, K., Yang, J., Luo, K., & Ma, X. (2024). An umbrella review of socioeconomic status and cancer. *Nature Communications*, 15(1).

<https://doi.org/10.1038/s41467-024-54444-2>

Lima, S. M., Kehm, R. D., & Terry, M. B. (2021). Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns. *EClinicalMedicine*, 38, 100985.

<https://doi.org/10.1016/j.eclinm.2021.100985>

Loibl, S., Poortmans, P., Morrow, M., Denkert, C., & Curigliano, G. (2021). Breast cancer. *The Lancet*, 397(10286), 1750–1769. [https://doi.org/10.1016/s0140-6736\(20\)32381-3](https://doi.org/10.1016/s0140-6736(20)32381-3)

Lousdal, M. L., Kristiansen, I. S., Møller, B., & Støvring, H. (2016). Effect of organised mammography screening on stage-specific incidence in Norway: population study. *British Journal of Cancer*, 114(5), 590–596. <https://doi.org/10.1038/bjc.2016.8>

MacLeod, K. E., Kamruzzaman, L., & Musselwhite, C. (2022). Transport and health equity, social inclusion and exclusion. *Journal of Transport & Health*, 27, 101543. <https://doi.org/10.1016/j.jth.2022.101543>

Marmot, M., Friel, S., Bell, R., Houweling, T. A., & Taylor, S. (2008). Closing the gap in a generation: health equity through action on the social determinants of health. *The Lancet*, 372(9650), 1661–1669. [https://doi.org/10.1016/s0140-6736\(08\)61690-6](https://doi.org/10.1016/s0140-6736(08)61690-6)

Martin, D., Miller, A. P., Quesnel-Vallée, A., Caron, N. R., Vissandjée, B., & Marchildon, G. P. (2018). Canada’s universal health-care system: achieving its potential. *The Lancet*, 391(10131), 1718–1735. [https://doi.org/10.1016/s0140-6736\(18\)30181-8](https://doi.org/10.1016/s0140-6736(18)30181-8)

McDonald, J. T., & Kennedy, S. (2004). Insights into the ‘healthy immigrant effect’: health status and health service use of immigrants to Canada. *Social Science & Medicine*, 59(8), 1613–1627. <https://doi.org/10.1016/j.socscimed.2004.02.004>

Mottram, R., Knerr, W. L., Gallacher, D., Fraser, H., Al-Khudairy, L., Ayorinde, A., Williamson, S., Nduka, C., Uthman, O. A., Johnson, S., Tsertsvadze, A., Stinton, C., Taylor-Phillips, S., & Clarke, A. (2021). Factors associated with attendance at screening for breast cancer: a systematic review and meta-analysis. *BMJ Open*, 11(11), e046660. <https://doi.org/10.1136/bmjopen-2020-046660>

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J. L., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MOCA: a

brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society*, 53(4), 695–699. <https://doi.org/10.1111/j.1532-5415.2005.53221.x>

Nelson, H. D., Fu, R., Cantor, A., Pappas, M., Daeges, M., & Humphrey, L. (2016). Effectiveness of Breast Cancer Screening: Systematic review and meta-analysis to update the 2009 U.S. Preventive Services Task Force Recommendation. *Annals of Internal Medicine*, 164(4), 244. <https://doi.org/10.7326/m15-0969>

Nightingale, C. J., Mitchell, S. N., & Butterfield, S. A. (2018). Validation of the timed up and Go test for assessing balance variables in adults aged 65 and older. *Journal of Aging and Physical Activity*, 27(2), 230–233. <https://doi.org/10.1123/japa.2018-0049>

Ochieng, C. A., Minion, J. T., Turner, A., Blell, M., & Murtagh, M. J. (2021). What does engagement mean to participants in longitudinal cohort studies? A qualitative study. *BMC Medical Ethics*, 22(1). <https://doi.org/10.1186/s12910-021-00648-w>

OECD. (2023). *Public pensions*. Retrieved December 29, 2024, from <https://www.oecd.org/en/topics/public-pensions.html>

Popovic, A., & Huecker, M. R. (2023, June 20). *Study Bias*. StatPearls - NCBI Bookshelf. <https://www.ncbi.nlm.nih.gov/sites/books/NBK574513/>

Prowse, S. R., Brazzelli, M., & Treweek, S. (2024). What factors influence the uptake of bowel, breast and cervical cancer screening? An overview of international research. *European Journal of Public Health*, 34(4), 818–825. <https://doi.org/10.1093/eurpub/ckae073>

Raghupathi, V., & Raghupathi, W. (2020). The influence of education on health: an empirical assessment of OECD countries for the period 1995–2015. *Archives of Public Health*, 78(1). <https://doi.org/10.1186/s13690-020-00402-5>

Riboli, E., Hunt, K., Slimani, N., Ferrari, P., Norat, T., Fahey, M., Charrondière, U., Hémon, B., Casagrande, C., Vignat, J., Overvad, K., Tjønneland, A., Clavel-Chapelon, F., Thiébaud, A., Wahrendorf, J., Boeing, H., Trichopoulos, D., Trichopoulou, A., Vineis, P., . . . Saracci, R. (2002). European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutrition*, 5(6b), 1113–1124.

<https://doi.org/10.1079/phn2002394>

Richardson-Parry, A., Baas, C., Donde, S., Ferraiolo, B., Karmo, M., Maravic, Z., Münter, L., Ricci-Cabello, I., Silva, M., Tinianov, S., Valderas, J. M., Woodruff, S., & Van Vugt, J. (2023). Interventions to reduce cancer screening inequities: the perspective and role of patients, advocacy groups, and empowerment organizations. *International Journal for Equity in Health*, 22(1). <https://doi.org/10.1186/s12939-023-01841-6>

Rockwood, K. (2005). A global clinical measure of fitness and frailty in elderly people. *Canadian Medical Association Journal*, 173(5), 489–495.

<https://doi.org/10.1503/cmaj.050051>

Sanford, N. N., Sher, D. J., Butler, S., Xu, X., Ahn, C., D’Amico, A. V., Rebbeck, T., Aizer, A. A., & Mahal, B. A. (2019). Cancer screening patterns among current, former, and never smokers in the United States, 2010-2015. *JAMA Network Open*, 2(5), e193759.

<https://doi.org/10.1001/jamanetworkopen.2019.3759>

Sankaranarayanan, R. (2014). Screening for cancer in Low- and Middle-Income countries. *Annals of Global Health*, 80(5), 412. <https://doi.org/10.1016/j.aogh.2014.09.014>

Sarfati, D. (2019). *Why social inequalities matter in the cancer continuum*. Reducing Social Inequalities in Cancer: Evidence and Priorities for Research - NCBI Bookshelf.

<https://www.ncbi.nlm.nih.gov/books/NBK566166/>

Statistics Canada. (2022a, April 19). *Canadian Community Health Survey: Combined data, 2019/2020*. <https://www150.statcan.gc.ca/n1/daily-quotidien/220419/dq220419d-eng.htm>

Statistics Canada. (2022b, November 23). *Population - Canada at a Glance, 2023*. <https://www150.statcan.gc.ca/n1/pub/12-581-x/2023001/sec1-eng.htm>

Sun, J., Frick, K. D., Liang, H., Chow, C. M., Aronowitz, S., & Shi, L. (2024). Examining cancer screening disparities by race/ethnicity and insurance groups: A comparison of 2008 and 2018 National Health Interview Survey (NHIS) data in the United States. *PLoS ONE*, 19(2), e0290105. <https://doi.org/10.1371/journal.pone.0290105>

Tan, M. P. (2022a). Healthcare for older people in lower and middle income countries. *Age And Ageing*, 51(4). <https://doi.org/10.1093/ageing/afac016>

Tan, M. P. (2022b). Healthcare for older people in lower and middle income countries. *Age And Ageing*, 51(4). <https://doi.org/10.1093/ageing/afac016>

Tarasenko, Y. N., Fleming, S. T., & Schoenberg, N. E. (2013). The relationship between perceived burden of chronic conditions and colorectal cancer screening among Appalachian residents. *The Journal of Rural Health*, 30(1), 40–49. <https://doi.org/10.1111/jrh.12035>

Teague, S., Youssef, G., Macdonald, J., Sciberras, E., Shatte, A., Fuller-Tyszkiewicz, M., Greenwood, C., McIntosh, J., Olsson, C. A., & Hutchinson, D. (2018). Retention strategies in longitudinal cohort studies: a systematic review and meta-analysis. *BMC Medical Research Methodology*, 18(1). <https://doi.org/10.1186/s12874-018-0586-7>

Tetzlaff, F., Nowossadeck, E., Jansen, L., Michalski, N., Barnes, B., Kraywinkel, K., & Hoebel, J. (2023). Widening area-based socioeconomic inequalities in cancer mortality in

Germany between 2003 and 2019. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-45254-5>

The Lancet Oncology. (2024). Curbing the climb in cancer incidence. *The Lancet Oncology*, 25(5), 529. [https://doi.org/10.1016/s1470-2045\(24\)00217-1](https://doi.org/10.1016/s1470-2045(24)00217-1)

Trapani, D., Sandoval, J., Aliaga, P. T., Ascione, L., Giachetti, P. P. M. B., Curigliano, G., & Ginsburg, O. (2023a). Screening programs for Breast Cancer: Toward Individualized, Risk-Adapted Strategies of Early Detection. *Cancer Treatment and Research*, 63–88. https://doi.org/10.1007/978-3-031-33602-7_3

Trapani, D., Sandoval, J., Aliaga, P. T., Ascione, L., Giachetti, P. P. M. B., Curigliano, G., & Ginsburg, O. (2023b). Screening programs for Breast Cancer: Toward Individualized, Risk-Adapted Strategies of Early Detection. *Cancer Treatment and Research*, 63–88. https://doi.org/10.1007/978-3-031-33602-7_3

UN. Population Division. (2017). *Population Facts*. Retrieved December 29, 2024, from https://www.un.org/en/development/desa/population/publications/pdf/popfacts/PopFacts_2017-1.pdf?utm_source=chatgpt.com

UN. Population Division. (2020). World Population Ageing 2020 : Highlights : Living arrangements of Older Persons. In *United Nations Digital Library System*. Retrieved December 29, 2024, from <https://digitallibrary.un.org/record/3898412/?v=pdf>

UN. Population Division. (2022). World Population Prospects 2022: Summary of Results. In <https://digitallibrary.un.org/record/3989515?v=pdf>. Retrieved December 29, 2024, from <https://www.un.org/development/desa/pd/content/World-Population-Prospects-2022>

United Nations. (2015). *Transforming our world: the 2030 Agenda for Sustainable Development*. <https://sdgs.un.org/2030agenda>

Vaccarella, S., Georges, D., Bray, F., Ginsburg, O., Charvat, H., Martikainen, P., Brønnum-Hansen, H., Deboosere, P., Bopp, M., Leinsalu, M., Artnik, B., Lorenzoni, V., De Vries, E., Marmot, M., Vineis, P., Mackenbach, J., & Nusselder, W. (2022). Socioeconomic inequalities in cancer mortality between and within countries in Europe: a population-based study. *The Lancet Regional Health - Europe*, 25, 100551. <https://doi.org/10.1016/j.lanepe.2022.100551>

Van Alten, S., Domingue, B. W., Faul, J., Galama, T., & Marees, A. T. (2024). Reweighting UK Biobank corrects for pervasive selection bias due to volunteering. *International Journal of Epidemiology*, 53(3). <https://doi.org/10.1093/ije/dyae054>

Vandenberghe, D., & Albrecht, J. (2019). The financial burden of non-communicable diseases in the European Union: a systematic review. *European Journal of Public Health*, 30(4), 833–839. <https://doi.org/10.1093/eurpub/ckz073>

Vang, Z., Sigouin, J., Flenon, A., & Gagnon, A. (2015). *The Healthy Immigrant Effect in Canada: A Systematic Review*. Scholarship@Western. <https://ir.lib.uwo.ca/pclrc/vol3/iss1/4/>

Vos, T., Lim, S. S., Abbafati, C., Abbas, K. M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abolhassani, H., Aboyans, V., Abrams, E. M., Abreu, L. G., Abrigo, M. R. M., Abu-Raddad, L. J., Abushouk, A. I., . . . Murray, C. J. L. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, 396(10258), 1204–1222. [https://doi.org/10.1016/s0140-6736\(20\)30925-9](https://doi.org/10.1016/s0140-6736(20)30925-9)

Walji, L. T., Murchie, P., Lip, G., Speirs, V., & Iversen, L. (2021). Exploring the influence of rural residence on uptake of organized cancer screening – A systematic review of international literature. *Cancer Epidemiology*, 74, 101995.

<https://doi.org/10.1016/j.canep.2021.101995>

Western, M. J., & Malkowski, O. S. (2022). Associations of the Short Physical Performance Battery (SPPB) with Adverse Health Outcomes in Older Adults: A 14-Year Follow-Up from the English Longitudinal Study of Ageing (ELSA). *International Journal of Environmental Research and Public Health*, 19(23), 16319. <https://doi.org/10.3390/ijerph192316319>

WHO. (2010, May 16). *Cancer - Screening and early detection*.

<https://www.who.int/europe/news-room/fact-sheets/item/cancer-screening-and-early-detection-of-cancer>

WHO. (2015). World Report on Ageing and Health. In

<https://www.who.int/publications/i/item/9789241565042/>.

<https://www.who.int/publications/i/item/9789241565042/>

WHO. (2019). *Social determinants of health*. https://www.who.int/health-topics/social-determinants-of-health#tab=tab_1

WHO. (2020a). *Healthy ageing and functional ability*. <https://www.who.int/news-room/questions-and-answers>. <https://www.who.int/news-room/questions-and-answers/item/healthy-ageing-and-functional-ability>

WHO. (2020b). *UN Decade of Healthy Ageing: Plan of Action (2021-2030)*. Retrieved December 29, 2024, from <https://www.who.int/westernpacific/publications/m/item/un-decade-of-healthy-ageing-plan-of-action-2021-2030>

WHO. (2023a). *Global Breast Cancer Initiative Implementation Framework: Assessing, strengthening and scaling up of services for the early detection and management of breast cancer*. <https://www.who.int/publications/i/item/9789240065987>

WHO. (2023b, September 16). *Noncommunicable diseases*. Retrieved December 29, 2024, from <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>

Wilkins, C. H. (2018). Effective engagement requires trust and being trustworthy. *Medical Care*, 56(Suppl 1), S6–S8. <https://doi.org/10.1097/mlr.0000000000000953>

Zielonke, N., Gini, A., Jansen, E. E., Anttila, A., Segnan, N., Ponti, A., Veerus, P., De Koning, H. J., Van Ravesteyn, N. T., Heijnsdijk, E. A., Veerus, P., Anttila, A., Heinävaara, S., Sarkeala, T., Cañada, M., Pitter, J., Széles, G., Voko, Z., Minozzi, S., . . . Priaulx, J. (2020a). Evidence for reducing cancer-specific mortality due to screening for breast cancer in Europe: A systematic review. *European Journal of Cancer*, 127, 191–206. <https://doi.org/10.1016/j.ejca.2019.12.010>

Zielonke, N., Gini, A., Jansen, E. E., Anttila, A., Segnan, N., Ponti, A., Veerus, P., De Koning, H. J., Van Ravesteyn, N. T., Heijnsdijk, E. A., Veerus, P., Anttila, A., Heinävaara, S., Sarkeala, T., Cañada, M., Pitter, J., Széles, G., Voko, Z., Minozzi, S., . . . Priaulx, J. (2020b). Evidence for reducing cancer-specific mortality due to screening for breast cancer in Europe: A systematic review. *European Journal of Cancer*, 127, 191–206. <https://doi.org/10.1016/j.ejca.2019.12.010>