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Influence of metabolic disorders on chronic disease burden and health outcomes

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ABSTRACT

Chronic diseases represent an escalating global health burden, with metabolic dysfunction and systemic inflammation emerging as central and interconnected biological drivers across multiple organ systems. This thesis investigates the relationship between metabolic-inflammatory dysregulation and chronic disease through three complementary studies addressing treatment response, diagnostic precision, and disease outcomes in severe asthma, neurocognitive decline, and non-alcoholic fatty liver disease respectively.

Study 1 examined whether obesity modulates the long-term effectiveness of biologic therapy in severe asthma in a real-world cohort of nearly 2,100 patients from the SANI registry. Despite presenting with a clinically more complex profile obese patients achieved sustained and comparable improvements across all key outcomes, including exacerbation frequency, asthma control, lung function, and clinical remission, over up to four years of follow-up. These findings provide strong evidence that biologic therapy remains equally effective regardless of BMI.

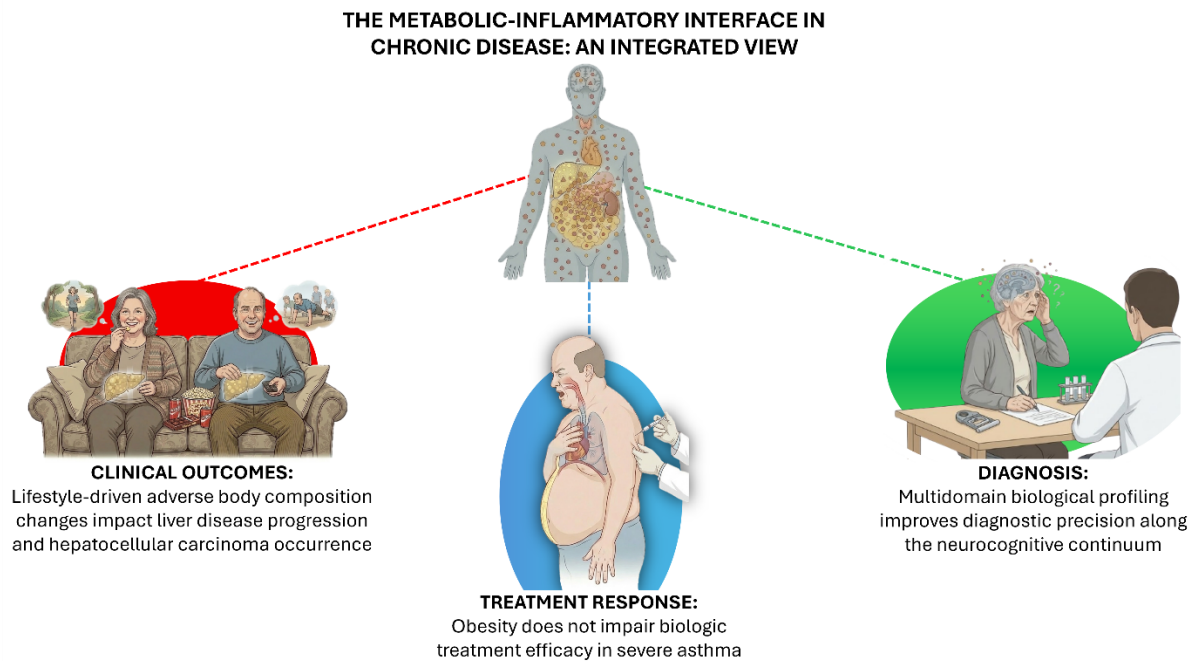
Study 2 investigated whether a multidimensional biological-cognitive approach could improve diagnostic discrimination along the neurocognitive continuum in approximately 100 geriatric outpatients spanning five diagnostic categories from Subjective Cognitive Complaint to Major Neurocognitive Disorder. A progressive Firth-penalized logistic regression model sequentially integrating neuropsychological performance, body composition, metabolic markers, and inflammatory cytokine profiling produced consistent discriminative gains across five clinically relevant pairwise comparisons. The inflammatory cytokine component provided the largest biological increment for the most diagnostically challenging early-stage comparisons, suggesting that peripheral inflammatory dysregulation may represent a biological signature of the earliest neurocognitive transitions. Multidimensional scaling and hierarchical cluster analysis identified a biologically structured two-dimensional profile space and two discrete biological phenotypes mapping coherently onto clinical diagnosis, supporting a shift toward comprehensive biological-cognitive patient characterisation in geriatric cognitive medicine.

Study 3 investigated the impact of pandemic-related lifestyle changes on clinical outcomes in a prospectively monitored NAFLD cohort. Lockdown-imposed reductions in physical activity and

increases in caloric intake were associated with worsening anthropometric, biochemical, and body composition parameters alongside accelerated liver disease progression and a marked increase in hepatocellular carcinoma occurrence. Body compartment alterations emerged as the main independent predictor of HCC risk, beyond established factors including liver stiffness, identifying body composition as a dynamic prognostic modifier in NAFLD.

Collectively, these studies provide convergent evidence that metabolic status is not a passive comorbidity but an active biological determinant of disease expression, diagnostic classification, and clinical outcomes.

GRAPHICAL ABSTRACT.



1. INTRODUCTION

1.1 Chronic diseases as a global public health challenge

The significant epidemiological shifts observed in recent decades have been accompanied by profound changes in the global disease landscape reflecting a transition from infectious to chronic conditions, driven by improved control of communicable diseases, population ageing, urbanisation, and the widespread adoption of unhealthy lifestyles. In response to these transformations, scientific research has adapted by focusing its efforts on altering the trajectories of diseases once deemed incurable and profound advances in public health measures, medical technologies, and acute care have dramatically reduced mortality from infectious diseases, maternal causes, and early-life conditions. These achievements have resulted in a substantial increase in life expectancy across all regions of the world [1]–[3]. However, increased longevity has also led to prolonged exposure to behavioural, metabolic, and environmental risk factors, fundamentally reshaping the global distribution of disease.

Chronic non-communicable diseases (NCDs) are long-lasting medical conditions that are not transmitted from person to person, typically resulting from a combination of genetic, behavioural, and environmental factors, and often leading to long-term health complications representing the most significant public health challenge of contemporary societies.

Current global evidence demonstrates that chronic diseases now dominate population health profiles, accounting for the majority of health loss worldwide. NCDs have become the predominant challenge for global health in the 21st century accounting for nearly two-thirds of deaths worldwide and represent the main causes of poor health, disability, and premature mortality in high- and middle-income countries [2],[4].

This dominance is observed not only in high-income countries but increasingly in low- and middle-income settings, where chronic diseases coexist with residual infectious burdens, producing complex and heterogeneous epidemiological landscapes [2],[5],[6]. Moreover, chronic diseases directly affect health-care budgets, labour productivity, and macroeconomic performance; the global cost of chronic

disease has been estimated to reach US$47 trillion by 2030, reflecting both direct medical expenditure and losses in productivity and human capital [6].

Despite this, investment and development assistance for NCD prevention and control remain disproportionately low relative to their contribution to global disease burden, particularly in low-resource settings [5],[6].

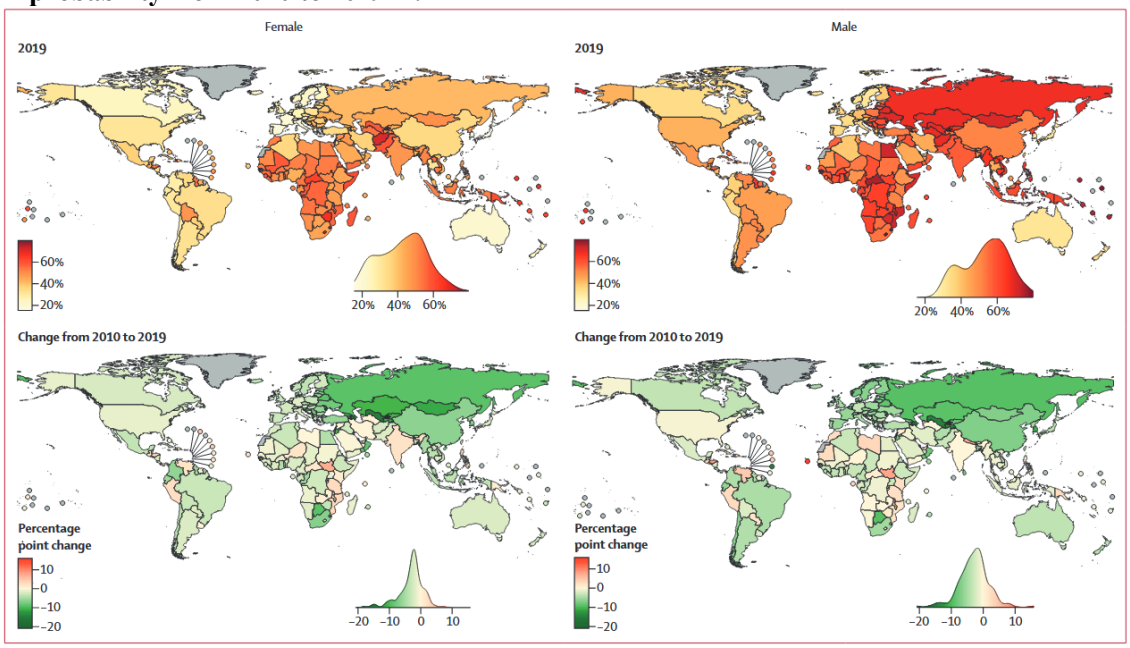
1.2 Burden of chronic diseases: morbidity, mortality, and disability

The growing global burden of chronic non-communicable diseases on mortality has significantly reshaped public health priorities over recent decades. While progress in controlling infectious diseases has led to dramatic declines in mortality from communicable diseases, maternal causes, and early-life conditions, NCDs have become the leading cause of death worldwide. In 2019, NCDs were responsible for 42 million of the 57 million deaths globally, with 27 million deaths occurring in individuals under the age of 80, marking a substantial shift towards diseases that are primarily influenced by lifestyle factors, metabolic health, and ageing [6].

In recent years, significant improvements have been observed in age-specific mortality rates across the globe, with reductions ranging from 27.1% to 35.0% in most countries between 2000 and 2023. These reductions reflect the positive impact of advances in healthcare, disease prevention, and public health policies.

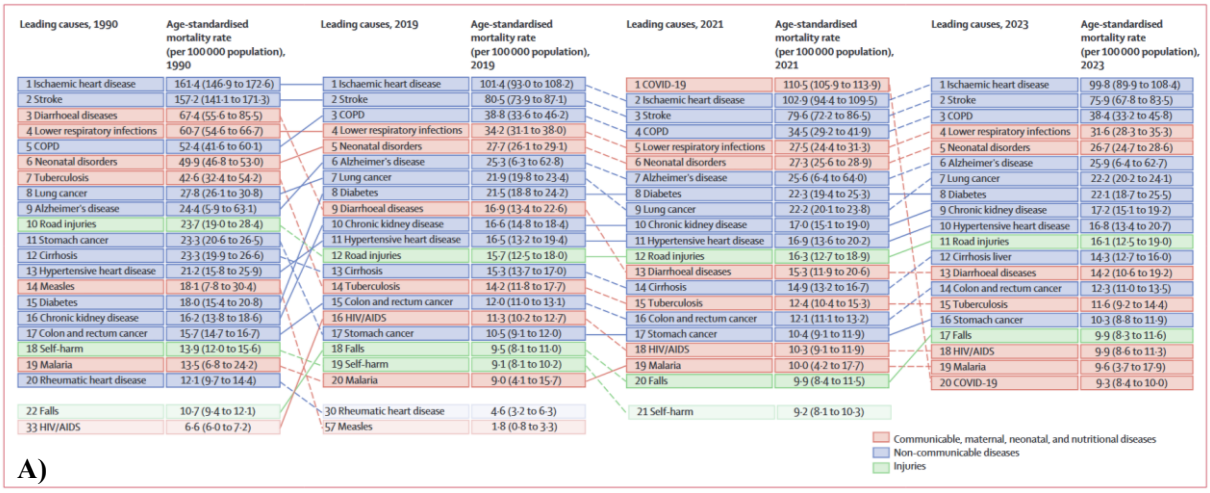
However, this general decline masks significant disparities by region, sex, and cause of death. Notably, in high-income regions such as North America and Europe, the decline in mortality rates from NCDs slowed down in the most recent decade (2010-2019), with some countries even experiencing reversals of earlier progress. In contrast, regions in sub-Saharan Africa and South Asia are seeing increasing mortality rates from NCDs, exacerbating the challenge of managing both communicable and non-communicable diseases simultaneously [7]. This disparity in mortality reduction highlights the complexity of addressing NCDs globally and the continued importance of targeting the key risk factors that underlie these diseases (Figure 1).

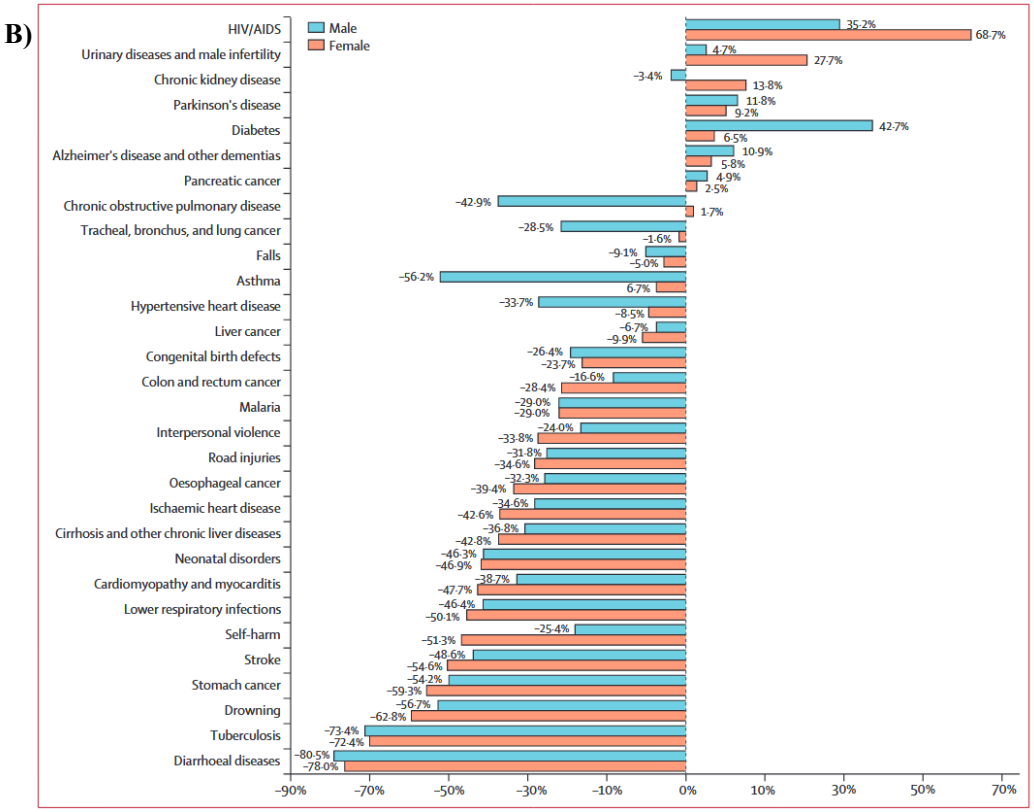
Figure 1. Probability of dying from an NCD between birth and age 80 years in 2019 and change in probability from 2010 to 2019 [6].



Cardiovascular diseases, including ischaemic heart disease, and stroke continue to be the leading causes of age-standardised mortality worldwide. While the age-standardised mortality rates for these diseases have decreased over time, the absolute number of deaths from NCDs continues to rise due to demographic shifts, particularly population ageing. In fact, the global age-standardised mortality rate for ischaemic heart disease declined from 161.4 deaths per 100,000 in 1990 to 99.8 deaths per 100,000 in 2023, and for stroke, mortality decreased from 157.2 deaths per 100,000 in 1990 to 75.9 deaths per 100,000 in 2023. Moreover, in this context, the COVID-19 pandemic temporarily shifted mortality patterns, with the virus causing an abrupt rise in mortality rates, but in 2023, it dropped to being the 20th leading cause of death globally, with NCDs once again occupying the top spots [6]. Despite these improvements, the increase in absolute mortality from NCDs underscores the critical challenges posed by the growing number of older adults who are living with chronic diseases, which contribute not only to premature mortality but also to long-term disability and dependence on healthcare services [8] (Figure 2).

Figure 2. A) Leading causes of global deaths and age-standardised mortality rate per 100, 000 population for all sexes combined, 1990, 2019, 2021, and 2023. B) Percentage change in global age-standardised mortality rate from 1990 to 2023 among the leading 30 causes of death, for males and females [7].





In recent years, there has been a noticeable increase in the mean age at death from chronic diseases globally, reflecting improvements in healthcare and disease management, particularly in high-income countries. For example, in Switzerland, the mean age of death from ischaemic heart disease in 2023 was 88.4 years, compared to 61.2 years in South Sudan, reflecting stark disparities in health outcomes.

Similar patterns are observed for lung cancer and chronic kidney disease, where the age at death differs widely depending on the country and the healthcare system in place. These variations highlight not only the increasing age at death for chronic diseases but also the substantial disparities in life expectancy based on socio-economic factors, healthcare access, and disease burden [7],[8].

Socio-economic status plays a critical role in shaping mortality rates, with individuals in lower socio-economic groups often experiencing higher risks of mortality from both communicable and non-communicable diseases. This disparity is influenced by access to healthcare, living conditions, education, and other structural factors that affect health outcomes with many countries still face significant challenges from infectious diseases, creating a "double burden" of disease. This phenomenon, observed in regions such as sub-Saharan Africa and South Asia, exacerbates the difficulty in addressing both communicable and non-communicable diseases, which require different approaches to prevention and treatment. NCDs mortality in these regions is often compounded by limited healthcare infrastructure and resources, which are insufficient to manage the increasing burden of chronic diseases effectively [7].

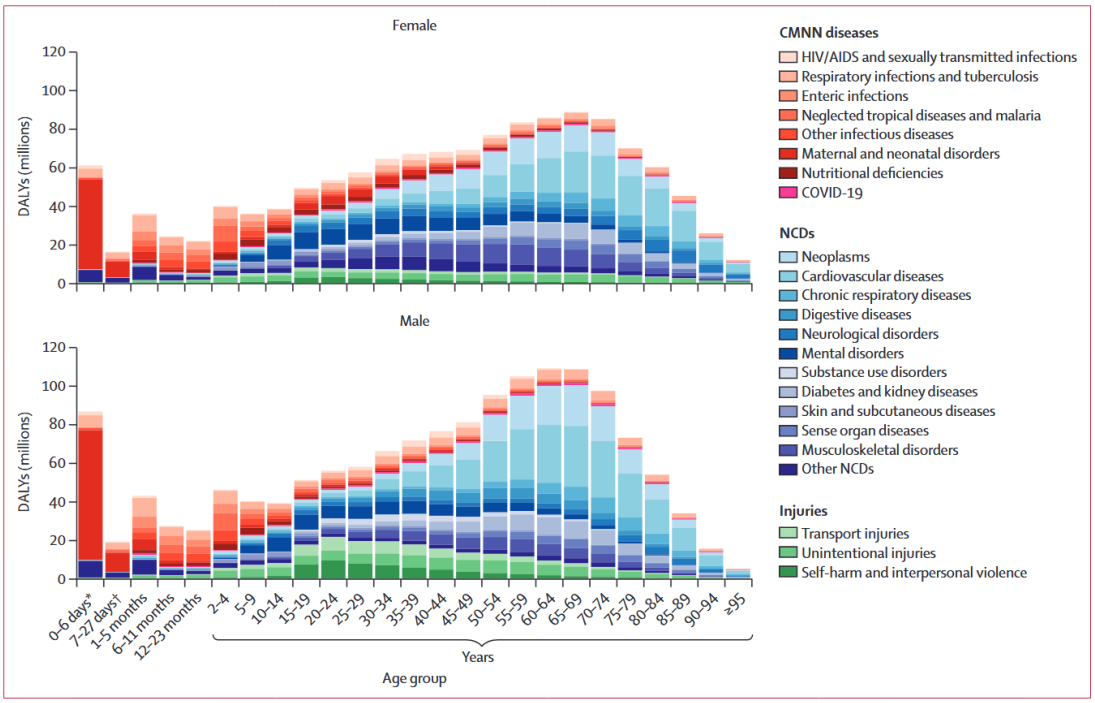
Addressing NCDs mortality requires a comprehensive approach that includes strengthening healthcare systems, improving access to prevention and treatment, and addressing the broader social determinants of health. Despite progress in high-income regions, the continued increase in NCD-related deaths, particularly in working-age populations, underscores the importance of early intervention and prevention efforts to curb the rise in mortality from chronic diseases globally. The success in reducing NCDs mortality, as seen in regions like North America and Western Europe, offers valuable lessons for countries struggling with increasing NCD-related mortality.

The burden of chronic non-communicable diseases is increasingly reflected not just in mortality, but in rising morbidity and disability. The Global Burden of Disease (GBD) study uses Disability-Adjusted Life Years (DALYs) to measure the overall health burden, combining both Years of Life Lost (YLLs) due to premature death and Years Lived with Disability (YLDs). In 2023, NCDs accounted for nearly two-thirds of global DALYs, with leading contributors such as cardiovascular diseases, stroke, diabetes, and chronic respiratory diseases. These conditions contribute significantly

to long-term disability, leading to greater functional impairments and healthcare dependency particularly in adults and older populations [4],[9].

Despite global age-standardised DALY rates decreased by about 13%, signalling improvements in health outcomes from 2010 to 2023 the absolute number of DALYs continued to rise by 6%, mainly due to population growth and ageing. This trend is particularly evident for NCDs, where DALYs counts increased from 1.45 billion in 2010 to 1.80 billion in 2023. The increase in YLDs, especially for diseases such as diabetes, chronic kidney disease, and mental health disorders, highlights the growing impact of chronic diseases on long-term health and quality of life. As these conditions often lead to functional limitations and dependency, healthcare systems worldwide face escalating challenges in managing chronic disease burden [1],[4] (Figure 3).

Figure 3. The distribution of global DALYs across age and sex for GBD causes in 2023 [4].



One of the key indicators of the rising morbidity linked to chronic diseases is the widening gap between life expectancy and healthy life expectancy. While people are living longer, much of this additional time is spent in ill health, contributing to prolonged disability. In 2023, ischaemic heart disease and stroke were the leading causes of age-standardised DALYs globally. Although the mean

age at death for these diseases has increased, particularly in high-income regions, the overall disability burden continues to rise, primarily due to the increasing rates of chronic conditions like diabetes and chronic kidney disease, which lead to long-term functional impairments.

The global disability burden from NCDs is also influenced by environmental and lifestyle factors, such as air pollution, poor diet, and lack of physical activity, which contribute to metabolic risks like high blood pressure and obesity. These factors have become key drivers of chronic diseases, further exacerbating the strain on healthcare systems, particularly in regions with limited resources. In low- and middle-income countries, where healthcare infrastructure is often inadequate, rising disability from NCDs is overwhelming healthcare systems that struggle to provide long-term care and treatment [7].

Economic analyses indicate that chronic diseases consume the majority of health-care spending around three-quarters in the United States, and impose large indirect costs through lost productivity and caregiving demands [1],[2]. With population ageing and rising prevalence of key risk factors, future projections suggest that an increasing proportion of life expectancy gains will be spent with disability from chronic conditions, underscoring the importance of prevention and effective long-term management [4],[5],[9].

1.3 Multimorbidity and complexity of chronic disease management

Multimorbidity, conventionally defined as the coexistence of two or more chronic conditions within the same individual, is a hallmark of population ageing and a central challenge for contemporary health care systems [10]. Its prevalence increases sharply with age, affecting between 55% and 98% of individuals over 60 years, and accounting for up to 80% of consultations in primary care and nearly all encounters in geriatric medicine [11]–[13]. These figures highlight how multimorbidity has become the dominant clinical scenario in later life, reflecting cumulative exposure to biological, behavioural, and social risk factors across the life course.

The coexistence of multiple chronic conditions substantially increases clinical complexity. Chronic diseases do not simply accumulate in an additive manner; rather, they interact through shared pathophysiological pathways, treatment effects, and functional consequences, resulting in health risks

that exceed the sum of individual conditions [11],[13]. Multimorbidity is associated with complex pharmacological regimens, polypharmacy, increased health care utilisation, and reduced quality and length of life. These features challenge clinicians who are predominantly trained within single-disease and specialty-based frameworks and place pressure on health care systems that struggle to provide comprehensive assessments, coordinated treatments, and integrated care pathways for individuals with multiple chronic conditions [13].

Beyond its overall prevalence, multimorbidity is characterised by marked heterogeneity. Epidemiological studies consistently show that chronic diseases tend to cluster within individuals in non-random patterns, with cardiometabolic, neuropsychiatric, and musculoskeletal clusters most frequently identified across populations [14]. However, substantial variability exists across studies, with numerous additional clusters described and considerable differences in their composition. While part of this heterogeneity reflects methodological variation, longitudinal evidence indicates that disease clusters are dynamic and evolve over time, influenced by ageing processes, disease progression, and mortality selection, features that cannot be adequately captured by cross-sectional designs [11],[13].

Longitudinal analyses following individuals over extended periods demonstrate that multimorbidity trajectories are not static but involve transitions between clusters with distinct clinical and prognostic profiles [11]. Some individuals present with relatively mild and unspecific disease patterns and remain stable for prolonged periods, whereas others progress toward more severe clusters dominated by cardiovascular or neuropsychiatric conditions. These trajectories are associated with differences in functional status, treatment burden, and prognosis, underscoring the relevance of person-centred and trajectory-based approaches to understanding and managing multimorbidity.

The management of multimorbidity is further complicated by important gaps in the evidence base. Older adults with multiple chronic conditions are frequently excluded from randomised clinical trials, resulting in limited guidance for clinical decision-making in real-world settings [12]. Clinical practice guidelines, largely designed around single diseases, often fail to address treatment prioritisation, competing risks, treatment burden, and feasibility in the context of co-occurring conditions, particularly among older adults [14]. This contributes to uncertainty in care delivery and fragmented clinical management.

From a health system perspective, multimorbidity is associated with sharply increasing health care costs, which rise disproportionately with each additional chronic condition, as well as distinct patterns of resource utilisation compared with single-disease populations. These trends have major implications for health care financing and service organisation, particularly in the context of demographic ageing and the projected global increase in complex multimorbidity over the coming decades [12],[13]. The growing prevalence of multimorbidity in both high-income and low- and middle-income countries further exposes the limitations of disease-centred models of care in addressing the needs of ageing populations.

1.4 Determinants of chronic disease burden

The burden of chronic diseases is shaped by a complex interplay of demographic, socioeconomic, behavioural, and structural determinants operating across the life course. These factors influence not only the risk of developing chronic conditions, but also the age of onset, patterns of disease clustering, severity, disability, and access to effective prevention and care.

Demographic change, particularly population ageing, represents a major driver of the global chronic disease burden. Age is consistently the strongest predictor of many high-burden chronic conditions, including cardiovascular disease, diabetes, obesity, chronic kidney disease, and several cancers [15]. As life expectancy increases, a growing proportion of the population survives to ages at which chronic diseases accumulate, leading to rising prevalence of both single chronic conditions and multimorbidity [5],[9],[16].

Beyond ageing, chronic disease burden is deeply structured by the social determinants of health, defined as the conditions in which people are born, grow, work, live, and age, and the broader social, economic, and political forces shaping daily life. These determinants, including income, education, occupation, housing, neighbourhood environments, environmental exposures, and experiences of discrimination, have a causal influence on the onset, progression, and outcomes of chronic diseases across the life course.

Lower socioeconomic status is consistently associated with higher prevalence of chronic diseases, earlier onset, greater severity, and poorer outcomes [5],[16],[17]. Large population-based studies show

that individuals with lower income or educational attainment are more likely to develop multiple chronic conditions, whereas those with stable insurance coverage and regular access to primary care experience lower disease burden [15]. Importantly, multimorbidity tends to occur earlier and with greater complexity in deprived populations, reinforcing health inequalities and placing disproportionate strain on under-resourced health systems [5],[16],[18].

Education emerges as a particularly strong determinant of chronic disease risk. Evidence from multi-country cohorts shows that lower educational attainment is associated with substantially higher risks of cardiovascular disease and other chronic conditions, even after adjustment for lifestyle factors, suggesting that social disadvantage operates through multiple material, psychosocial, and biological pathways [15],[17]. These patterns reflect a well-established social gradient in health, whereby each step down the socioeconomic hierarchy is associated with progressively worse health outcomes.

Sex and gender further structure chronic disease burden and interact with socioeconomic position across the life course. Evidence from the Global Burden of Disease Study 2021 demonstrates persistent differences between females and males across the top 20 global causes of disease burden over the past three decades [19]. Overall, males experience a higher total disease burden, with higher age-standardised DALY rates for most mortality-driven conditions, including cardiovascular diseases, chronic respiratory diseases, liver disease, road injuries, and COVID-19. In contrast, females consistently show higher DALY rates for morbidity-driven conditions, including low back pain, depressive and anxiety disorders, headache disorders, other musculoskeletal conditions, Alzheimer's disease and other dementias, and HIV/AIDS.

These sex differences emerge early in life and widen with age. Adolescence represents a critical period during which biological changes intersect with gender socialisation, shaping long-term exposure to behavioural and psychosocial risk factors. Over the life course, females accumulate higher levels of morbidity and disability, despite longer life expectancy, resulting in a greater proportion of life spent in ill health. In ageing populations, this pattern has important implications for health system planning, particularly for long-term and community-based care.

Ethnicity and race intersect with socioeconomic and gender-based inequalities to further shape chronic disease burden. Racialised and minoritised populations in many high-income countries experience

disproportionate burden due to the combined effects of socioeconomic disadvantage, structural racism, environmental exposures, and unequal access to quality care; these pre-existing disparities were starkly highlighted during the COVID-19 pandemic [2],[18],[20].

A relatively small set of modifiable lifestyle and behavioural factors accounts for a substantial proportion of the global chronic disease burden. Physical inactivity, unhealthy diet, tobacco use, and harmful alcohol consumption are consistently identified as the principal behavioural drivers of major non-communicable diseases, associated disability, and reduced quality of life [1],[2],[17].

However, behavioural risk factors are not randomly distributed within populations. They are strongly shaped by social and environmental conditions, including housing quality, neighbourhood safety, availability of healthy foods, occupational demands, economic insecurity, and exposure to marketing of unhealthy products [17],[18]. As a result, unhealthy behaviours tend to cluster in socially disadvantaged groups, amplifying existing health inequalities.

Smoking provides a clear example of this interaction between social context and behavioural risk. While the biological mechanisms linking tobacco use to cancer, cardiovascular disease, and chronic respiratory disease are well established, smoking itself is a socially patterned behaviour, influenced by social networks, stress, and socioeconomic disadvantage [17],[18]. Over time, smoking prevalence has declined among higher socioeconomic groups while becoming increasingly concentrated among those lower on the social ladder, contributing to widening disparities in chronic disease burden.

Dietary behaviours show similar patterns. Diets characterised by high intake of ultra-processed foods, added sugars, and saturated fats and low consumption of fruits, vegetables, and whole grains are more common in populations facing economic constraints and limited access to healthy food environments, promoting obesity, diabetes, and cardiovascular disease across the life course [1],[2]. Physical inactivity is likewise influenced by urban design, occupational structures, and access to safe spaces for exercise.

Evidence from population surveys indicates that engagement in health-promoting behaviours, such as regular physical activity and preventive healthcare use, is associated with lower risk of chronic diseases, whereas obesity and metabolic abnormalities substantially increase risk [3]. Despite this strong evidence base, investment in primary prevention and health promotion remains limited relative

to treatment spending, underscoring the importance of addressing behavioural risk factors within their broader social and structural context [1],[2],[5],[18].

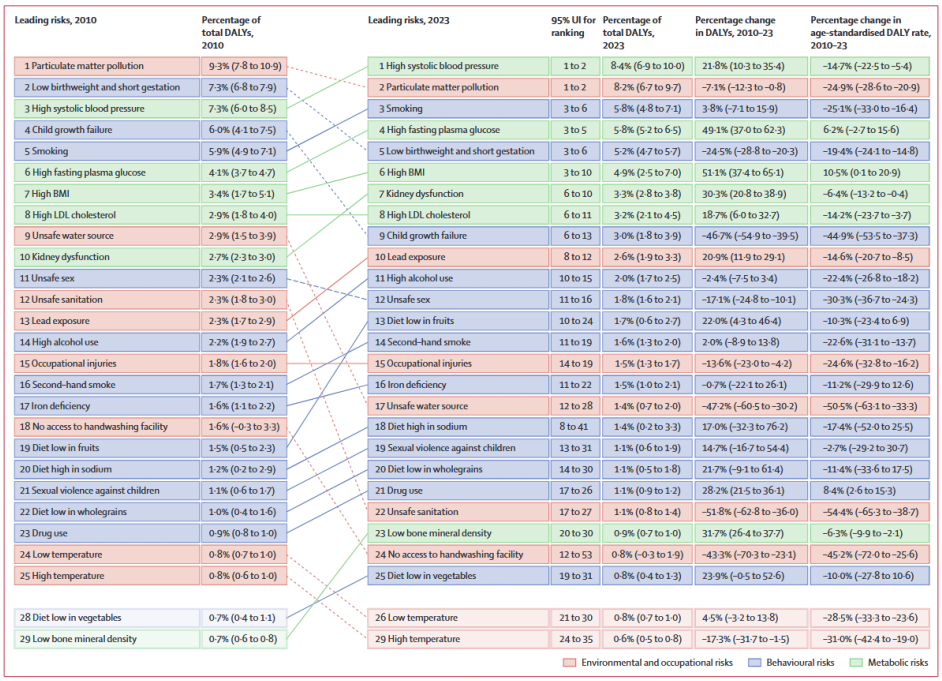
Together, these demographic, socioeconomic, and behavioural determinants shape the distribution and progression of chronic diseases across populations by influencing long-term exposure to upstream risk factors. Among these, metabolic alterations emerge as a central biological pathway through which social and behavioural influences are translated into chronic disease onset, progression, and adverse health outcomes, warranting focused consideration in the following section.

1.5 Metabolic disorders within the chronic disease framework

Metabolic disorders represent a central component of the contemporary chronic disease burden and a key pathway linking behavioural, social, and biological determinants to adverse health outcomes. In 2023, nearly half of all global DALYs were attributable to the 88 risk factors quantified within the GBD framework, amounting to 1.27 billion DALYs, or 45.5% of total global health loss. Among level 1 risk categories, behavioural risk factors accounted for the largest share of attributable DALYs (28.9%), followed by metabolic risks (18.1%) and environmental and occupational risks (16.0%) [4].

At a more detailed level, metabolic risk factors accounted for a substantial share of the global disease burden. High systolic blood pressure was the leading risk factor worldwide in 2023, contributing 8.4% of total DALYs. Other key metabolic risks, including high fasting plasma glucose, high body mass index, elevated LDL cholesterol, and kidney dysfunction, were also among the leading contributors to global DALYs, highlighting the central role of metabolic dysfunction in the development of chronic diseases globally [4] (Figure 4).

Figure 4. Leading 25 GBD risk factors by attributable DALYs as a percentage of total DALY counts (2010 and 2023), and percentage change in attributable DALY counts and age-standardised DALY rates from 2010 to 2023 [4].



The relevance of metabolic risk factors increases progressively from adolescence onward. While early childhood disease burden is mainly driven by nutritional and environmental risks, metabolic factors begin to play an important role from young adulthood. In individuals aged 15–49 years, high body mass index and high systolic blood pressure already rank among the leading risk factors. From midlife onwards, metabolic risks become the main drivers of disease burden, with obesity, hypertension, impaired glucose regulation, dyslipidaemia, and kidney dysfunction consistently contributing to chronic disease burden [4].

Temporal trends further emphasise the growing relevance of metabolic risks. Between 2010 and 2023, global DALYs counts attributable to metabolic risks increased by more than 30%, in contrast to declining DALYs counts attributable to behavioural and environmental and occupational risks [4]. Although age-standardised DALYs rates attributable to metabolic risks declined modestly over the same period, this reduction was substantially smaller than that observed for behavioural risks or environmental risks. Notably, age-standardised burden attributable to high body mass index increased

globally, and burden attributable to high fasting plasma glucose showed a non-significant upward trend, reflecting the expanding impact of obesity and dysglycaemia in ageing populations [4].

At the biological level, metabolic disorders represent shared pathways through which diverse exposures exert effects across multiple chronic diseases. Evidence from genetic, metabolomic, and epidemiological studies indicates that alterations in lipid and glucose metabolism, liver function, and low-grade systemic inflammation act as common mediators linking behavioural and environmental exposures to a wide range of non-communicable diseases [12].

Longitudinal population-based studies of older adults further support this framework, demonstrating that cardiometabolic risk factors including diabetes, obesity, dyslipidaemia, and hypertension precede and predict transitions into more complex multimorbidity clusters, particularly those characterised by cardiovascular and neuropsychiatric conditions [13]. These clusters are consistently associated with the highest levels of disability, functional impairment, and health loss over time, highlighting the central role of metabolic disorders in shaping long-term health trajectories beyond single-disease outcomes.

1.6 Systemic inflammation as a shared biological pathway

Inflammation is a highly conserved biological response essential for host defence and tissue repair. In its acute form, inflammation is a tightly regulated, time-limited process initiated by infectious agents or tissue injury through the recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) by innate immune receptors [21]–[23]. Acute inflammation is characterised by rapid activation of innate immune cells, local production of pro-inflammatory mediators, and subsequent engagement of an active resolution phase. This resolution phase is mediated by specialised pro-resolving mediators (SPMs), including lipoxins, resolvins, protectins, and maresins, which promote clearance of apoptotic cells, limit neutrophil recruitment, suppress cytokine production, and restore tissue homeostasis [22].

Chronic inflammation arises when these resolution mechanisms fail. Systemic low-grade chronic inflammation is not driven by acute infection but by persistent sterile stimuli, including metabolic stress, cellular damage, and environmental exposures, leading to sustained immune activation in the absence of overt clinical signs [21],[22]. This state represents a qualitative shift from protective

inflammation to a maladaptive process that progressively impairs tissue structure and function and exerts cumulative damage over time [22],[23].

A large body of experimental, clinical, and epidemiological evidence indicates that systemic chronic inflammation represents a shared biological substrate across a broad spectrum of chronic non-communicable diseases [21],[23]. Persistent inflammatory signalling contributes to disease initiation, accelerates progression, and increases disease co-occurrence, thereby providing a mechanistic basis for multimorbidity [12],[14]. Unlike disease-specific pathways, chronic inflammation operates across multiple organ systems, amplifying vulnerability to cardiometabolic, respiratory, neurodegenerative, renal, musculoskeletal, autoimmune, and malignant conditions.

Inflammation-related biomarkers, most consistently C-reactive protein, interleukin-6, tumour necrosis factor-α, and composite inflammatory indices, have been repeatedly associated with higher disease burden, disability, and mortality across populations [14],[21]. Importantly, these associations persist even after adjustment for traditional risk factors, supporting inflammation as an independent and integrative driver of chronic disease burden.

At the molecular level, systemic chronic inflammation is sustained by continuous exposure to endogenous danger signals generated by metabolic and cellular stress. Metabolism-associated molecular patterns (MAMPs) including free fatty acids, cholesterol crystals, advanced glycation end products, oxidised lipoproteins, uric acid, mitochondrial DNA, and glucose, activate innate immune pathways such as NF-κB signalling and inflammasome complexes [21],[22]. These pathways promote persistent production of pro-inflammatory cytokines and chemokines, leading to widespread immune–metabolic dysregulation.

Oxidative stress plays a central role in perpetuating this process. Excessive production of reactive oxygen species (ROS) induces cellular damage and further release of inflammatory signals, establishing positive feedback loops in which inflammation and oxidative stress mutually reinforce each other [22]. Over time, these processes contribute to fibrosis, mitochondrial dysfunction, endoplasmic reticulum stress, impaired insulin signalling, cellular senescence, and tissue remodelling, all of which are hallmarks of chronic disease progression.

Systemic chronic inflammation increases with advancing age, a phenomenon widely described as *inflammaging* [21]. Ageing is associated with higher circulating levels of inflammatory mediators, increased expression of inflammation-related genes, and accumulation of senescent cells exhibiting a senescence-associated secretory phenotype (SASP), characterised by sustained release of pro-inflammatory cytokines, growth factors, and proteases [12],[21]. These age-related changes reflect lifelong accumulation of molecular damage, metabolic stress, and environmental exposures rather than chronological ageing alone.

Importantly, inflammaging displays substantial inter-individual variability. Differences in lifestyle, diet, obesity, physical inactivity, microbiome composition, chronic infections, environmental pollutants, and broader social and environmental determinants contribute to heterogeneity in inflammatory burden across individuals and populations [21],[22]. This variability helps explain divergent trajectories of chronic disease risk, multimorbidity, and functional decline in later life.

1.7 Metainflammation: the immunometabolic interface

Metabolic dysfunction represents one of the most robust and biologically coherent links between systemic chronic inflammation and the burden of chronic diseases across the life course. Metabolic risk factors, including obesity, insulin resistance, impaired glucose regulation, dyslipidaemia, hypertension, and kidney dysfunction, are not merely downstream consequences of chronic disease, but active drivers of persistent inflammatory activation [12],[21],[22],[24]. These conditions generate a sustained pro-inflammatory milieu through the continuous release of metabolic danger signals that activate innate immune pathways and disrupt metabolic homeostasis.

From a metabolic and evolutionary perspective, chronic inflammation is increasingly recognised not simply as a consequence of metabolic disease, but as a causal and amplifying driver of metabolic dysfunction. Evolutionary pressures have favoured biological systems optimised to defend against energy scarcity rather than energy excess. As a result, organisms possess robust and redundant mechanisms that promote energy storage, glucose production, and protection against hypoglycaemia, traits that conferred survival advantage under conditions of nutritional limitation [24]. In modern environments characterised by chronic nutrient surplus, physical inactivity, and prolonged exposure

to obesogenic conditions, these same adaptive mechanisms become maladaptive, predisposing to obesity, insulin resistance, and type 2 diabetes. Within this framework, immune mediators, particularly cytokines, can be conceptualised as metabolic hormones, reflecting the deep evolutionary integration of immune and metabolic systems and giving rise to the concept of *metainflammation* (also termed *metaflammation*), defined as chronic, low-grade, metabolically driven inflammation [24].

At the tissue level, adipose tissue plays a central role in this process. In obesity, adipose tissue undergoes profound structural and functional remodelling characterised by adipocyte hypertrophy, hypoxia, immune cell infiltration, particularly macrophages, and altered secretion of adipokines and cytokines [22]. This shift transforms adipose tissue from an energy storage organ into an active inflammatory site, promoting systemic insulin resistance and chronic low-grade inflammation. Lipid exposure drives polarisation of adipose tissue macrophages toward a pro-inflammatory phenotype, which interferes with insulin signalling through cytokines such as tumour necrosis factor (TNF) and interleukin-1β (IL-1β) [21],[24]. Free fatty acids, cholesterol crystals, and adipocyte-derived mediators act as metabolism-associated molecular patterns, activating NF-κB signalling, Toll-like receptor pathways, and inflammasome complexes, thereby directly linking nutrient excess to inflammatory cascades and metabolic dysregulation characteristic of metainflammation.

The inflammasome pathway represents a critical molecular node in this immunometabolic interface. Activation of inflammasome components leads to the maturation of IL-1β and IL-18, cytokines with well-established roles in impairing insulin sensitivity and β-cell function. Experimental models consistently demonstrate that disruption of inflammasome signalling confers protection against diet-induced insulin resistance, while clinical studies in humans show that pharmacological inhibition of IL-1 signalling improves glycaemic control and preserves β-cell function [21],[24]. These findings provide compelling evidence for a causal role of metainflammation in metabolic disease, rather than a purely associative relationship.

Impaired glucose metabolism further amplifies inflammatory signalling. Chronic hyperglycaemia promotes the formation of advanced glycation end products and increased oxidative stress, both of which activate inflammatory cascades and endothelial dysfunction [22]. These processes contribute to the development and progression of type 2 diabetes, metabolic-associated fatty liver disease, and

microvascular complications, while simultaneously increasing susceptibility to cardiovascular and renal disease. Importantly, inflammatory activation in metabolic disorders is bidirectional: inflammation worsens insulin sensitivity and lipid metabolism, while metabolic dysregulation sustains inflammation, creating a self-reinforcing cycle of immunometabolic impairment [21],[24].

At the cellular and organelle level, chronic inflammation is reinforced by oxidative stress, endoplasmic reticulum stress, and mitochondrial dysfunction. Persistent inflammatory signalling disrupts cellular energy handling and amplifies immune activation through positive feedback loops that converge on key regulatory pathways [22],[24]. These pathways integrate immune sensing with metabolic regulation and exhibit pleiotropic, context-dependent effects, underscoring both the complexity of metainflammatory regulation and the limitations of therapeutic strategies targeting single inflammatory mediators.

Chronic inflammation also plays a key role in the fibrotic remodelling observed in metabolic diseases. Persistent inflammatory signalling promotes activation of profibrotic pathways, particularly through transforming growth factor-β–mediated fibroblast activation and extracellular matrix deposition [22]. In the liver, this mechanism drives progression from steatosis to steatohepatitis and cirrhosis; in the kidney, it underlies diabetic and hypertensive nephropathy; and in the cardiovascular system, it contributes to myocardial fibrosis and vascular stiffening. These fibrotic changes represent irreversible structural damage that links metainflammation to long-term functional decline.

Ageing introduces an additional layer of complexity through the phenomenon of inflammaging. Age-associated accumulation of senescent cells exhibiting a senescence-associated secretory phenotype sustains low-grade inflammatory signalling over time, further increasing susceptibility to insulin resistance, dyslipidaemia, sarcopenia, and frailty [14],[21],[24]. This chronic inflammatory background helps explain the progressive clustering of metabolic abnormalities across the life course.

At the population level, the convergence of metabolic risk factors and chronic inflammation helps explain the dominant contribution of metabolic disorders to multimorbidity and disability in ageing societies [12],[14]. Biomarker studies consistently show that inflammatory markers cluster with metabolic abnormalities and are strongly associated with the number, severity, and progression of chronic conditions, as well as with functional impairment [14].

Collectively, this evidence positions chronic inflammation, and more specifically metainflammation, not as a secondary epiphenomenon of metabolic disease, but as a central immunometabolic mechanism through which evolutionary constraints, lifestyle exposures, genetic susceptibility, and metabolic stress converge to accelerate the development, interaction, and progression of chronic diseases across multiple organ systems [21]–[24].

1.8 Metabolic status as a modifier of chronic disease expression and treatment response

Metabolic status functions as a critical modifier of health outcomes across a wide spectrum of chronic diseases, influencing disease susceptibility, progression, severity, and response to treatment. Metabolic disorders such as obesity, insulin resistance, type 2 diabetes, dyslipidaemia, and metabolic-associated fatty liver disease alter systemic physiology in ways that extend well beyond classical metabolic endpoints, reshaping inflammatory tone, immune function, vascular integrity, and tissue repair capacity [23],[25]–[28]. As a result, the presence of metabolic disease can amplify the burden of otherwise distinct chronic conditions, contributing to heterogeneity in clinical trajectories and outcomes [24],[29].

At the biological level, chronic metabolic dysfunction modifies disease expression through sustained low-grade inflammation, oxidative stress, endothelial dysfunction, and altered cellular energetics. These processes influence key pathogenic pathways shared across cardiovascular, renal, respiratory, neurodegenerative, musculoskeletal, autoimmune, and malignant diseases [30],[31]. For example, insulin resistance and hyperglycaemia exacerbate vascular inflammation and fibrosis, accelerating atherosclerosis, chronic kidney disease, and heart failure, while obesity-related inflammatory signalling alters immune surveillance and tissue microenvironments, affecting cancer progression and outcomes [22],[23]. In this context, metabolic disease acts not simply as a comorbidity, but as a biological amplifier that lowers resilience and narrows physiological reserve [32]–[34].

Metabolic status also plays a pivotal role in shaping therapeutic effectiveness across chronic diseases [35]. Obesity, diabetes, and related metabolic alterations influence drug pharmacokinetics and

pharmacodynamics by modifying drug absorption, distribution, metabolism, and clearance [36],[37]. Changes in body composition, hepatic metabolism, renal function, plasma protein binding, and inflammatory enzyme activity can lead to underexposure or toxicity for commonly used therapies, including cardiovascular drugs, immunomodulators, anticoagulants, chemotherapeutic agents, and biologics [12],[24]. Chronic inflammation further interferes with drug response by altering receptor signalling, downstream pathway sensitivity, and immune–drug interactions, contributing to variable treatment efficacy across metabolically distinct patient populations.

Conversely, pharmacological treatments for chronic diseases can exert profound effects on metabolic status, with important implications for long-term outcomes. Several widely used drug classes including glucocorticoids, antipsychotics, immunosuppressants, and some cardiovascular therapies, can worsen insulin resistance, promote weight gain, dyslipidaemia, or hepatic steatosis, thereby reinforcing metainflammatory pathways and increasing cardiometabolic risk [38]. In contrast, many cornerstone metabolic therapies, such as metformin, GLP-1 receptor agonists, SGLT2 inhibitors, and lifestyle interventions, exhibit pleiotropic anti-inflammatory and organ-protective effects that extend beyond glycaemic control, improving outcomes in cardiovascular, renal, and inflammatory diseases [21],[23],[39]–[41].

These bidirectional interactions highlight that treatment efficacy in chronic disease cannot be fully understood without accounting for underlying metabolic context. Identical therapies may yield divergent benefits or harms depending on metabolic health, inflammatory burden, and tissue-level metabolic stress. This helps explain observed variability in clinical trial outcomes, differential drug responsiveness, and disparities in real-world effectiveness across populations with differing metabolic profiles.

Taken together, metabolic status emerges as a central modifier of chronic disease outcomes, operating through interconnected immunometabolic, vascular, and pharmacological mechanisms. Recognising metabolic disease as an active determinant of disease behaviour and treatment response rather than a passive comorbidity, supports a shift toward more integrated, metabolism-informed approaches to chronic disease management, risk stratification, and therapeutic decision-making across the life course.

2. RATIONALE AND CONCEPTUAL FRAMEWORK OF THE PHD THESIS

The global burden of chronic diseases, largely driven by metabolic dysfunction, represents an escalating public health challenge with profound implications for individuals, healthcare systems, and society. As the prevalence of chronic conditions continues to rise, particularly in aging populations, understanding the complex interplay between metabolic disturbances and chronic disease development has become increasingly important for improving both therapeutic strategies and preventive approaches.

This PhD thesis investigates these issues through three distinct studies, each devoted to a different facet of the relationship between metabolic dysfunction and chronic disease: treatment response, improvement of diagnostic approaches, and the role of metabolic factors in shaping clinical outcomes.

Asthma, a common chronic respiratory disease affecting millions worldwide, is frequently complicated by comorbidities such as obesity. The first study focuses on therapeutic effectiveness, investigating whether a metabolic factor, specifically obesity, may modulate the efficacy and outcomes of biologic treatments in severe asthma. In the context of the growing use of biologic therapies, this study explores the extent to which obesity may influence treatment response, with particular relevance for long-term disease control and personalized management.

The second study investigates cognitive health in an elderly outpatient cohort, with a primary focus on improving diagnostic accuracy through the evaluation of metabolic-inflammatory patterns. In the context of population aging and the growing prevalence of cognitive decline and neurocognitive disorders, the study integrates clinical, functional, and biological data to assess whether inflammatory and metabolic profiles may enhance the precision of diagnostic classification and support the distinction between normal cognitive aging and more advanced forms of impairment. By identifying biologically meaningful patterns, the study seeks to contribute to earlier, more accurate, and clinically informative diagnostic pathways.

The third study explores the impact of metabolic factors, particularly lifestyle habits, on disease outcomes in non-alcoholic fatty liver disease. Since body composition, diet, and physical activity are closely linked to the progression of liver disease, this study investigates how acute lifestyle changes

may influence clinical outcomes, including the progression of liver damage and the occurrence of hepatocellular carcinoma. In this way, the study provides insight into the prognostic relevance of modifiable metabolic factors and their potential role as targets for prevention and treatment.

Collectively, these studies highlight different but interconnected aspects of chronic disease management: therapeutic efficacy modulated by a metabolic factor, diagnostic improvement through metabolic-inflammatory profiling, and the impact of metabolic and lifestyle-related factors on disease outcomes. By examining these complementary perspectives, the thesis aims to contribute to a more personalized and effective approach to the prevention, diagnosis, and treatment of chronic diseases.

3. STUDY 1 - CLINICAL OUTCOMES AND REMISSION TRAJECTORIES IN OBESE AND NON-OBESE PATIENTS WITH SEVERE ASTHMA TREATED WITH BIOLOGICS: A LONGITUDINAL COHORT STUDY FROM THE SEVERE ASTHMA NETWORK ITALY (SANI) REGISTRY.

3.1 Background

Asthma is a major global health issue that affects approximately 270 million of individuals worldwide, both in childhood and adulthood. As a chronic condition characterized by airway inflammation, hyper-responsiveness, and periodic obstruction, asthma leads to significant morbidity and mortality and it is associated with frequent exacerbations, poor quality of life, and high healthcare costs, placing a considerable burden on healthcare systems globally [42]–[44]. Asthma manifests in varying degrees of severity, ranging from mild, intermittent symptoms to severe, persistent cases that require more intensive management and treatment.

3.1.1. *Epidemiology of asthma*

The global burden of asthma is substantial. Asthma is a major global health issue that affects approximately 270 million individuals worldwide, both in childhood and adulthood, and causing approximately 450,000 deaths per year. As a chronic condition characterized by airway inflammation, hyper-responsiveness, and periodic obstruction, asthma leads to frequent exacerbations, poor quality of life, and high healthcare costs, placing a considerable burden on healthcare systems globally [42]. Regional differences in asthma rates are significant and are influenced by various factors such as socioeconomic status, healthcare access, air quality, and lifestyle factors. In high-income countries, asthma prevalence has been on the rise for several decades, primarily due to changes in lifestyle, urbanization, and exposure to environmental pollutants. Conversely, in many low-income countries, asthma rates remain relatively low, though there are signs of rising incidence due to urban migration, increased pollution, and dietary changes [42],[44],[45].

3.1.2 *Risk Factors for asthma*

Risk factors associated with asthma prevalence include heredity, exposure to tobacco smoke, viral exposure, air pollution, obesity, genetic risk factors, sex (boys have a higher risk than girls before puberty) stress, some allergen exposures, urbanisation, scarcity of beneficial microbial exposures, socioeconomic status and occupational exposures in adults.

Asthma is a highly heritable condition, with genetic factors accounting for up to 80% of asthma cases, especially in childhood-onset asthma. Specific genetic markers, such as those found in the IL18R1, HLA genes have been associated with asthma susceptibility. These genetic factors influence the immune response, making certain individuals more prone to developing asthma when exposed to environmental triggers [42],[43].

The interaction between genetic and environmental factors is complex, and asthma can be thought of as a "gene-environment" interaction disease. Environmental exposures, such as air pollution or allergens, can trigger asthma in genetically predisposed individuals, leading to the onset of the disease.

The role of diet in asthma development is increasingly recognized. Diets rich in fruits and vegetables, and particularly those high in antioxidants, are associated with a reduced risk of developing asthma in children. Conversely, a diet high in processed foods and low in antioxidants may increase asthma risk.

Regular physical activity is also protective against asthma, as it helps maintain healthy lung function and reduces inflammation. Socioeconomic status (SES) plays a crucial role in asthma prevalence, with individuals from lower SES backgrounds being more likely to develop asthma. These individuals are often exposed to more environmental risk factors, such as poor air quality and lack of access to healthcare, and may also experience higher levels of stress, further exacerbating asthma risk.

Obesity is a growing epidemic that is closely linked to asthma. The association between obesity and asthma is bidirectional: obesity increases the risk of developing asthma, and asthma exacerbations often lead to weight gain due to reduced physical activity and side effects from medication, particularly corticosteroids. Adipose tissue itself is an active immunologic organ, producing pro-inflammatory cytokines like leptin, which contribute to systemic inflammation and may worsen asthma symptoms [42]–[44],[46]. The relationship between obesity and asthma underscores the importance of addressing both conditions simultaneously in asthma management.

3.1.3. *Pathogenesis and Mechanisms of asthma*

Asthma is a chronic inflammatory disease of the airways, characterized by intermittent bronchospasm that causes symptoms such as wheezing, dyspnea, and cough. These symptoms are a result of airway inflammation, hyper-responsiveness, and mucus hypersecretion, all contributing to variable airflow obstruction. The underlying mechanisms driving asthma are heterogeneous, with various inflammatory pathways contributing to the clinical manifestations of the disease [42]–[44]. While asthma's immunological landscape is complex, the predominant mechanism involves Type 2 inflammation, which is central to its pathogenesis.

The immune response in asthma is multifaceted, with distinct patterns of airway inflammation observed between patients (Figure 5). The two most recognized inflammatory phenotypes are eosinophilic and neutrophilic, though other endotypic mechanisms remain less well understood. Advances in molecular inflammatory profiling have provided deeper insights into these mechanisms, yet the complexity of asthma inflammation suggests that multiple factors contribute to its development and progression [43],[44].

One of the key features of asthma pathophysiology is airway remodelling, a process that contributes to persistent airflow obstruction over time. Airway remodelling involves a combination of subepithelial fibrosis, basal membrane thickening, mucosal oedema, and hypertrophy of airway smooth muscle. These structural changes, although partially reversible with appropriate treatment, lead to a progressive decline in lung function. The thickening of the airway walls, along with smooth muscle hypertrophy and mucosal oedema, contributes to airway narrowing, further exacerbating the hyper-responsiveness of the airways [42],[43].

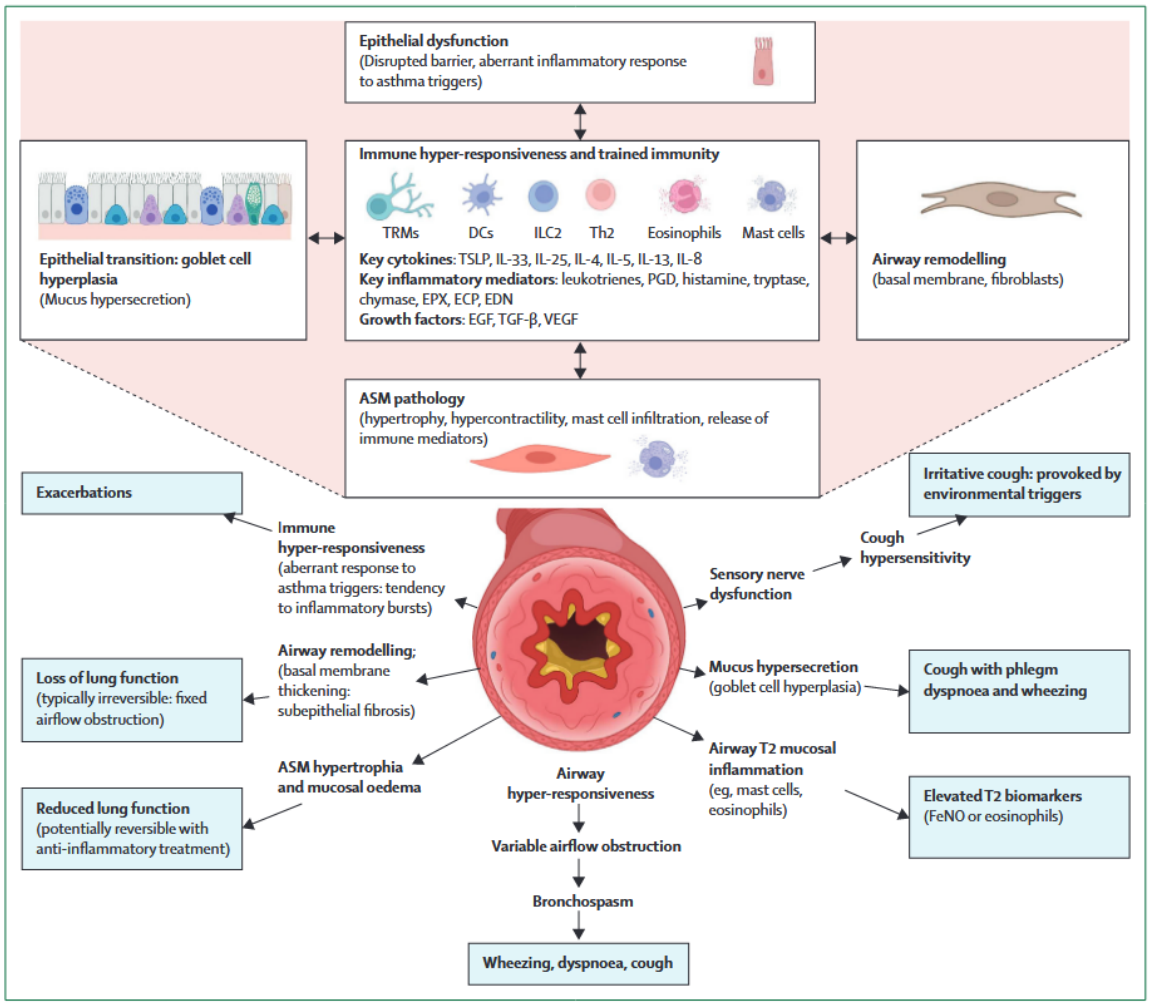
At the core of Type 2 inflammation in asthma is the activation of T-helper 2 (Th2) cells, eosinophils, mast cells, and group 2 innate lymphoid cells (ILC2). These immune cells play a central role in the inflammatory response, promoting both airway obstruction and hyper-responsiveness. Th2 cells and ILC2 cells release key cytokines such as IL-4, IL-5, and IL-13, which directly mediate allergic responses, including the production of IgE by B cells, the recruitment of eosinophils, and the activation of mast cells.

IL-4 and IL-13 are pivotal in driving the differentiation of B cells into IgE-producing cells, which are responsible for initiating allergic reactions upon exposure to environmental allergens. IL-5 plays a critical role in the recruitment and activation of eosinophils, which, in turn, release cytotoxic proteins that damage the airway epithelium and contribute to ongoing inflammation. Eosinophils are one of the primary effector cells in asthma, and their infiltration into the airway is a hallmark of the disease.

Mast cells, another key component of the immune system in asthma, release histamine and leukotrienes, which are potent bronchoconstrictors. Mast cell activation contributes to airway smooth muscle contraction, leading to bronchoconstriction and increased airway resistance, a hallmark of asthma exacerbations. Additionally, mast cells play a role in promoting airway hyper-responsiveness, which results in the heightened sensitivity of the airways to both specific and nonspecific triggers, such as allergens, exercise, and respiratory infections.

Thymic stromal lymphopoietin (TSLP), a cytokine released by the airway epithelium, serves as a critical initiator of the inflammatory cascade in asthma. TSLP acts as a "master switch," priming immune cells such as dendritic cells, Th2 cells, and ILC2 cells, and plays a significant role in the early stages of allergic asthma. This cytokine has been identified as an important mediator in the initiation and perpetuation of Type 2 inflammation, further driving the chronic inflammation seen in asthma [42],[43].

Figure 5. Key pathophysiological mechanisms of asthma and resulting disease components and clinical features [44].



Bottom: dyspnoea and wheezing are caused by variable airflow obstruction, reflecting airway smooth muscle hypercontractility. Top: a range of pathophysiological mechanisms interact to cause these clinical features of asthma. These factors might interact in different manners and with variation between patients. TRM, tissue resident memory cell; DC, dendritic cell; ILC2, innate lymphoid cell; Th2, T2 helper cells; TSLP, thymic stromal lymphopoietin; PGD; prostaglandins; EPX, eosinophil peroxidase; ECP, eosinophilic cationic protein; EDN, eosinophil-derived neurotoxin; EGF, epidermal growth factors; TGF-β, tumour growth factor β; VEGF, vascular endothelial growth factor; ASM, airway smooth muscle; FeNO, fractional exhaled nitric oxide.

3.1.4 *Diagnosis of asthma*

Criteria for the Initial Diagnosis of Asthma

The diagnosis of asthma in adults, adolescents, and children (aged 6–11 years) is based on a combination of clinical history, symptoms, and objective tests that confirm variable expiratory airflow limitation. The following criteria are commonly used to establish the diagnosis [47]:

1. History of Typical Variable Respiratory Symptoms

Asthma symptoms are typically intermittent and vary in intensity. Common features include:

- Wheezing, shortness of breath, chest tightness, and/or cough.
- Symptoms occur variably over time, often worsening at night or upon waking.
- Exercise, laughter, allergens, or cold air can trigger symptoms, and they often worsen during viral infections.

2. Confirmed Variable Expiratory Airflow Limitation

Airflow limitation in asthma is marked by excessive variability, which can be assessed using several tests. These include:

- *Positive Bronchodilator Responsiveness Test*:
    - Adults: An increase in FEV1 (Forced Expiratory Volume in 1 second) or FVC (Forced Vital Capacity) of ≥12% and ≥200 mL after inhalation of a bronchodilator (e.g., salbutamol). A higher increase (≥15% and ≥400 mL) is more indicative of asthma. If spirometry is unavailable, PEF (Peak Expiratory Flow) may be used, with an increase of ≥20% being a positive result.
    - Children: An increase in FEV1 of ≥12% predicted or an increase in PEF of ≥15% is considered positive.
    - These tests are typically performed 10–15 minutes after administration of 200–400 mcg salbutamol (or an equivalent bronchodilator).
- *Excessive Variability in Peak Expiratory Flow (PEF):*
    - Adults: An average daily diurnal variability in PEF >10%.
    - Children: An average daily diurnal variability in PEF >13% over two weeks.
    - This variability is typically measured using twice-daily PEF monitoring over a two-week period.
- *Increase in Lung Function with Treatment*:
    - Adults: A ≥12% increase in FEV1 (or ≥20% increase in PEF) after 4 weeks of daily inhaled corticosteroid (ICS) treatment.
    - Children: A ≥12% increase in FEV1 predicted (or ≥15% in PEF) after similar treatment.
- *Positive Bronchial Challenge Test*:

- Adults: A ≥20% fall in FEV1 from baseline with a standard dose of methacholine, hypertonic saline, or mannitol, or a >10% and >200 mL fall with an exercise challenge.
- Children: A >12% predicted fall in FEV1 (or >15% fall in PEF) with a standardized exercise challenge.
- If FEV1 decreases during the challenge, it is essential to check the FEV1/FVC ratio to confirm a genuine reduction, as other issues like inducible laryngeal obstruction may result in false readings.

- *Excessive Variation in Lung Function Between Visits*:
  - Adults: Variation in FEV1 of ≥12% and ≥200 mL (or in PEF ≥20%) between visits.
  - Children: Variation in FEV1 of ≥12% (or in PEF ≥15%) between visits.

These tests are essential for confirming the diagnosis of asthma, particularly when symptoms are intermittent, or other conditions might mimic asthma. The combination of variable symptoms and confirmatory tests, such as bronchodilator responsiveness and PEF monitoring, provides a comprehensive assessment for diagnosing asthma in clinical practice.

5. Definition and Criteria of Severe Asthma

Severe asthma is defined as asthma that requires high-dose treatment (GINA steps 4-5) to maintain control or requires systemic corticosteroids (CS) for more than 50% of the year to prevent it from becoming uncontrolled or remains uncontrolled despite optimal therapy. Uncontrolled asthma is characterized by at least one of the following [48]:

1. **Poor Symptom Control**:
   - ACQ (Asthma Control Questionnaire) consistently >1.5
   - ACT (Asthma Control Test) <20
   - Symptoms not well controlled according to NAEPP/GINA guidelines
2. **Frequent Severe Exacerbations**:
   - Two or more bursts of systemic CS (each >3 days) in the previous year
3. **Serious Exacerbations:**
   - At least one hospitalization, ICU stay, or mechanical ventilation in the previous year

4. **Airflow Limitation:**
    - FEV1 (Forced Expiratory Volume in 1 second) <80% of the predicted value after appropriate bronchodilator use
    - Reduced FEV1/FVC ratio (below the lower limit of normal)

Severe asthma may also be defined in cases where asthma worsens when tapering high doses of ICS, systemic CS, or additional biologics. This definition of severe asthma is important for guiding treatment decisions and understanding the severity of the disease in individual patients.

3.1.5 *Treatment of asthma*

The treatment of asthma has undergone significant evolution, shifting from a focus solely on alleviating symptoms to a more comprehensive and proactive strategy aimed at not only controlling inflammation and preventing exacerbations but also achieving deeper therapeutic goals, such as clinical remission. In this context, clinical remission refers to a state where patients experience complete symptom control, remain free from exacerbations, and can discontinue the use of systemic corticosteroids, all while maintaining stable lung function, typically defined as ≥80% of the predicted FEV1 [49]–[51]. This approach reflects a broader understanding of asthma management, focusing not just on symptom relief but on improving long-term outcomes, reducing disease progression, and optimizing the patient's quality of life.

Conventional asthma management involves a combination of inhaled corticosteroids (ICS), bronchodilators, and sometimes leukotriene modifiers, aimed at controlling inflammation and preventing bronchoconstriction. ICS are the cornerstone of asthma treatment, as they reduce airway inflammation, improve lung function, and prevent exacerbations. Long-acting beta-agonists (LABA) are typically used in combination with ICS to provide bronchodilation and improve symptom control. According to the Global Initiative for Asthma (GINA) guidelines, asthma management follows a stepwise approach, with therapy intensified depending on symptom severity and control. For mild asthma, low-dose ICS may be sufficient, but for moderate to severe asthma, higher doses of ICS or the addition of LABA, leukotriene modifiers, or other therapies are often required [44],[46],[47].

Severe Asthma

In patients with severe asthma, asthma remains poorly controlled despite high-dose ICS combined with LABA or other controllers. These patients often experience frequent exacerbations, poor quality of life, and significant limitations in daily activities. In such cases, oral corticosteroids are frequently used to control exacerbations, but prolonged use of OCS is associated with substantial risks, including bone loss, weight gain, and increased susceptibility to infections [47],[48],[50].

For patients who fail to respond to conventional therapies, biologic treatments targeting specific components of the immune system have emerged as a promising option.

Biologic Therapies

Biologic therapies have revolutionized the treatment of severe asthma, particularly for patients with Type 2 inflammation. These therapies target specific cytokines, immune cells, and pathways that drive airway inflammation. Monoclonal antibodies targeting cytokines like IL-5, IL-4, IL-13, and TSLP have shown significant efficacy in reducing exacerbations, improving lung function, and decreasing the need for oral corticosteroids [51]–[53].

Anti-IL-5 and Anti-IL-5 Receptor Biologics

Mepolizumab, reslizumab, and benralizumab are monoclonal antibodies targeting IL-5 or the IL-5 receptor. IL-5 plays a key role in eosinophil activation, recruitment, and survival, making it a critical mediator in eosinophilic asthma. By inhibiting IL-5, these biologics reduce eosinophil-driven inflammation and improve asthma control. Mepolizumab and reslizumab have shown significant reductions in asthma exacerbations and improvements in lung function in patients with elevated eosinophils. Benralizumab, which targets the IL-5 receptor, also provides similar benefits, with studies showing that patients with eosinophilic asthma experience a substantial reduction in exacerbations.

Anti-IL-4 and Anti-IL-13 Biologics

Dupilumab is a monoclonal antibody that blocks the IL-4 receptor alpha (IL-4RA), which is shared by both IL-4 and IL-13. These cytokines play a central role in the allergic inflammation that underlies asthma. Dupilumab has been shown to reduce asthma exacerbations, improve lung function, and decrease the need for systemic corticosteroids, particularly in patients with moderate to severe asthma. Additionally, dupilumab has been shown to be effective in patients with comorbid conditions such as chronic rhinosinusitis with nasal polyps and atopic dermatitis.

Anti-TSLP Biologics

Tezepelumab, a monoclonal antibody targeting thymic stromal lymphopoietin (TSLP), has shown promise in treating severe asthma. TSLP is a key initiator of Type 2 inflammation and acts as a "master switch" for many of the inflammatory cascades involved in asthma. Tezepelumab's ability to inhibit TSLP has made it effective in treating a broad range of asthma phenotypes, including those with low eosinophilic involvement. Clinical trials have shown that tezepelumab significantly reduces asthma exacerbations and improves asthma control, making it a valuable treatment option for patients with severe, uncontrolled asthma.

3.1.6 *Obesity and asthma*

Despite significant advancements in asthma management, there remains considerable interindividual variability in treatment responses, and understanding the factors that influence remission continues to be a critical area of research [49]. One such factor that has garnered increasing attention is obesity [45]. Obesity is highly prevalent in patients with severe asthma, with estimates indicating that more than 50% of individuals in this group are obese [54]. Beyond being a risk factor for asthma development and poor disease control, obesity appears to define a distinct asthma phenotype. This phenotype is characterized by a diminished response to inhaled corticosteroids, reduced lung function, and increased healthcare utilization [54]. As a result, obesity significantly complicates asthma management. Recognizing its importance, several national and international guidelines, including the A2BCD guide, highlight the management of comorbidities like obesity as a central aspect of comprehensive asthma care [55].

Recent real-world studies and registry-based analyses have highlighted that higher body mass index (BMI) or obesity may act as negative predictors of clinical remission in patients undergoing biologic treatment, though the findings remain inconsistent [51],[53],[56],[57]. While patients with Type 2-high (T2-high) biomarkers generally have a greater likelihood of benefiting from biologics, obesity has been linked to a reduced chance of achieving remission, regardless of the specific biologic agent used [56]. Several factors could explain this association, including altered pharmacokinetics and impaired drug distribution due to excess adipose tissue, obesity-related systemic inflammation, and the presence of

overlapping comorbidities such as obstructive sleep apnea and gastroesophageal reflux disease [58]–[60]. These factors collectively contribute to the reduced effectiveness of biologic therapies in obese individuals.

3.2 Aims

The study aims to investigate the relationship between obesity and the efficacy of biologic therapies in asthma management, with a focus on evaluating:

1. Clinical outcomes:
    - Exacerbations requiring systemic corticosteroids
    - Emergency department visits
    - Hospitalizations
    - Unscheduled visits
2. Disease control and pulmonary function markers:
    - Asthma Control Test (ACT) scores
    - Asthma Quality of Life Questionnaire (AQLQ) scores
    - Percent predicted pre-bronchodilator Forced Expiratory Volume in 1 second (FEV1%)
3. Clinical remission

By exploring these outcomes, the study seeks to gain deeper insights into how obesity impacts the effectiveness of biologic treatments and its role in achieving asthma remission.

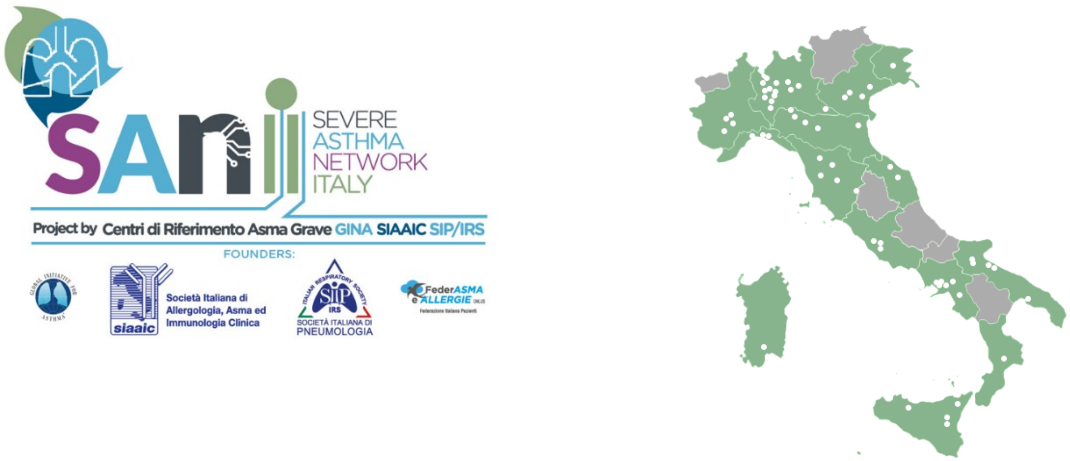
3.3 Methods

3.3.1 *Experimental design*

This retrospective, longitudinal study aimed to explore the efficacy of biologic therapies in patients with severe asthma. The study utilized data from the SANI registry [61], a nationwide network of specialized asthma centres across Italy, including patients aged ≥12 years who were enrolled between January 2017 and December 31, 2024 (Figure 6). Ethical approval for the collection, storage, and

analysis of anonymized data was granted, with informed consent obtained from all patients. The protocol is registered at ClinicalTrials.gov (ID NCT06625216; retrospectively registered on October 3, 2024).

Figure 6. Geographic distribution of referral Centres currently involved in SANI project [61].



The study evaluated both clinical outcomes (such as exacerbations, emergency visits, and hospitalizations) and disease control markers (such as ACT scores, AQLQ scores, and FEV1%). These outcomes were assessed longitudinally at multiple time points, with maximum follow-up depending on the specific outcome (up to 48 months). A before-and-after design was used for biologic-naïve patients, comparing their status before and after starting biologic therapy, while for patients already on biologic treatment, outcomes were assessed over time from registry entry with the preceding year serving as a reference.

3.3.2 *Study population*

The study population included patients with severe asthma enrolled in the SANI registry, a nationwide network of specialized asthma centres across Italy. The cohort comprised patients aged ≥12 years, with enrolment spanning from January 2017 to December 31, 2024.

To be eligible for inclusion, patients had to meet the ERS/ATS [48] and GINA [47] definitions of severe asthma, comply with the national reimbursement criteria for biologic therapy and have registry data available before or at the time of biologic initiation for at least one study domain.

Patients were classified as biologic-naïve if they had ≤1 month of biologic exposure at enrolment, and as on-treatment if they had >1 month of biologic exposure. Among the biologic-naïve group, 71 patients had initiated treatment within one month of enrolment (mean exposure 21.1 days [SD 9.5], range 2–31 days).

Baseline demographic data, including age, sex, BMI, smoking history, as well as clinical characteristics such as asthma disease onset and duration, biomarkers, and treatment history, were recorded. Obesity was defined as having a BMI ≥30 kg/m².

3.3.3 *Outcomes*

The outcomes evaluated in the study included exacerbations requiring systemic corticosteroids, emergency department visits, hospitalizations, unscheduled visits, Asthma Control Test (ACT) scores, Asthma Quality of Life Questionnaire (AQLQ) scores, percent predicted pre-bronchodilator Forced Expiratory Volume in 1 second (FEV1%), and clinical remission.

Clinical remission was defined based on the SANI Expert Consensus, with a slight modification to the lung function criterion by adding pre-bronchodilator FEV1 ≥80% predicted as part of stable lung function. Remission was defined as meeting the following criteria for at least 12 consecutive months: (1) no need for oral corticosteroid (OCS) treatment, (2) absence of asthma exacerbations or attacks, (3) no relevant asthma symptoms (ACT ≥20), and (4) stable lung function, with pre-bronchodilator FEV1 ≥80% predicted. Complete remission required all four criteria, while partial remission required the absence of OCS use plus any two of the remaining three criteria.

A T2-high profile was defined as having at least two of the following three biomarkers: FeNO ≥25 ppb, blood eosinophils ≥300 cells/μL, or total serum IgE ≥100 IU/mL.

3.3.4 *Statistical methods*

No formal sample size calculation was performed for this study; instead, all available registry data were utilized. Group characteristics were compared using Pearson's chi-square test for categorical variables and binary data, while Student's t-test was used for normally distributed continuous variables.

For non-normally distributed continuous variables and follow-up time comparisons, the Wilcoxon rank-sum test was employed.

To assess the associations between BMI and counts of exacerbations, hospitalizations, unplanned visits, and emergency department admissions, zero-inflated negative binomial (ZINB) models were used. These models accounted for overdispersion and the presence of excess zeros in the count data, ensuring a more accurate evaluation.

Mixed-effects models were applied to examine the relationships between BMI and outcomes such as the ACT, AQLQ scores, and FEV1%. These models incorporated both fixed and random effects to account for within-subject variability over time.

Kaplan–Meier curves were used to estimate the probability of achieving clinical remission over time, comparing obese versus non-obese patients. Groups were compared using the log-rank test. Since remission could only be observed after a minimum of 12 months of follow-up, the earliest time at which remission could be assigned was after 12 months. Missing data within the first 12 months such as patients not yet observed, loss to follow-up, or patients who had died, were examined to determine whether they reflected differential non-response between BMI categories (<30 vs. ≥30). This approach aimed to minimize attrition bias between groups.

Cox proportional hazards models were used to evaluate the relationship between BMI and partial or complete remission in patients who had not achieved remission at baseline. The proportional hazards assumption was tested using the Schoenfeld Residuals Test.

Given the substantial amount of missing data across treatment groups and BMI categories, sensitivity analyses using multiple imputation by chained equations (MICE) were conducted. This allowed for an assessment of the robustness of the primary findings, resulting in a larger analytic sample and increased statistical power.

Further sensitivity analyses modelled BMI as a continuous variable in both the ZINB and mixed-effects models. To aid in interpretation, post-estimation margins were used to derive predicted values at representative BMI levels, which were then plotted graphically. BMI was also modelled as a continuous variable in Cox regression.

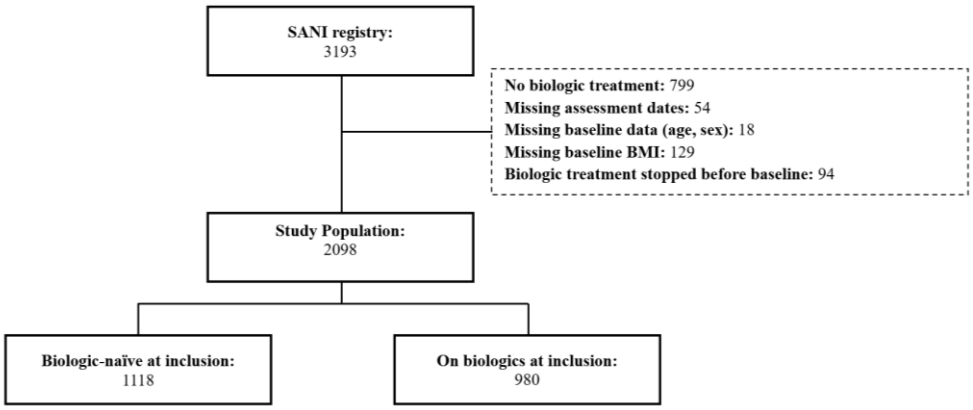
All models were adjusted for potential confounders, including age, sex, the presence of chronic rhinosinusitis with nasal polyps (CRSwNP), blood eosinophil count, FEV1 (% predicted), ACT score, and the number of days on biologic therapy at baseline. These adjustments were made based on literature evidence suggesting their potential influence on the outcomes.

Statistical analyses were performed using Stata version 15.0 SE (StataCorp, College Station, TX, USA).

3.4 Results

As of December 3, 2024, a total of 3193 patients had been enrolled in the SANI registry, with 2098 meeting all inclusion criteria. Of these, 1118 patients were biologic-naïve at inclusion (initiated biologic therapy at or after enrolment), and 980 patients were already receiving biologic treatment (on-treatment). The main reasons for exclusion included the absence of biologic initiation during the study period (n=799, controlled on high-dose inhaled therapy) and lack of biological exposure during follow-up (n=94). (Figure 7).

Figure 7. Study 1 - Flow-Chart.



On-treatment patients were a heterogeneous group, with varying durations of biologic exposure (mean 935,6 [SD 1111,6] days). As a result, baseline data (referring to the year preceding inclusion) were influenced by ongoing biologic therapy, with variability depending on the duration of treatment before enrolment. At baseline, 641 (31%) of patients were receiving anti-IgE therapy, 1151 (55%) anti-IL5/R, 248 (12%) anti-IL4/13, and 49 (2%) anti-TSLP, with no significant differences observed across BMI categories (Table 1).

Table 1. Distribution of Biologic Therapies at Inclusion According to BMI and Treatment Status (Biologic-Naïve vs. On Biologics)

	Biologic-naïve at inclusion				On biologics at inclusion			
	Total N=1118	**BMI<30** N=893	**BMI≥30** N=225	***P***	**Total** N=980	**BMI<30** N=800	**BMI≥30** N=180	***P***
Overall	1110	885	225	0·315	979	799	180	0·706
Omalizumab	223	170	53		418	339	79	
Mepolizumab	390	310	80		339	276	63	
Benralizumab	272	226	46		150	121	29	
Dupilumab	179	145	34		69	60	9	
Tezepelumab	46	34	12		3	3	0	

Distribution of biologic agents among patients naïve to biologics and those already on biologic therapy at inclusion, stratified by BMI (<30 vs ≥30). Data are presented as absolute numbers. P values indicate comparisons between BMI categories.

Baseline characteristics are summarized in Table 2. Patients were stratified by BMI (<30 vs ≥30) within both the biologic-naïve and on-treatment groups. Biologic-naïve patients were slightly younger than those already on biologics (median age 56 [IQR 48; 64] vs 58 [49; 65] years), with higher age in obese patients across both groups. Female sex was more prevalent in obese patients (38,0% vs 59,5% in naïve; 70,6% vs 60,6% in on-treatment). Asthma control and quality of life were worse in biologic-naïve patients compared to those already on biologics and were lower in obese patients in both groups. Specifically, ACT scores were 13,9 vs 15,4 in biologic-naïve (p<0·001); 18,1 vs 19,3 in on-treatment (p=0,025); AQLQ scores were 3,8 vs 4,0 in biologic-naïve (p=0,032); 4,7 vs 5,0 in on-treatment (p=0,073). Pre- and post-bronchodilator FEV1 did not differ by BMI.

The number of exacerbations per patient was comparable across BMI categories in both cohorts. However, the proportion of patients with ≥2 exacerbations in the previous year was higher among

biologic-naïve patients than on-treatment patients (56,0% vs 48,1%). Interpretation of this difference is limited by the absence of baseline exacerbation data and the heterogeneity in treatment duration in the on-treatment group. Among biologic-naïve patients, obesity was associated with a non-significant trend toward fewer individuals experiencing ≥2 events (51,4% vs 57,1%, p=0,17), whereas in the on-treatment cohort, the difference was statistically significant (38,9% vs 50,1%, p=0,006).

Daily prednisone-equivalent doses were slightly lower in on-treatment patients but did not differ by BMI (naïve, p=0,68; on-treatment, p=0,59). Biologic-naïve obese patients had lower FeNO levels than non-obese patients (23,5 vs 37,3 ppb, p=0,001). Blood eosinophil counts were significantly lower in obese patients (0,36 vs 0,43 $\times 10^9$/L; p=0,03). The prevalence of a T2-high profile was greater in non-obese biologic-naïve patients (p=0,023), while no differences by BMI were observed in the on-treatment group. Most patients tested positive for allergies to inhalants, with no BMI-related differences.

Comorbidities were more common in obese patients, particularly obstructive sleep apnea (OSAS), gastroesophageal reflux disease (GERD), cardiovascular disease, and type 2 diabetes (all p<0,001, except GERD in the on-treatment group). CRwNP was less frequent in obese patients.

Table 2. Baseline characteristics of the study population by BMI status.

	Biologic-naïve at inclusion				**On biologics at inclusion**			
	Total	**BMI<30**	**BMI≥30**	***P***	**Total**	**BMI<30**	**BMI≥30**	***P***
	N=1118	N=893	N=225		N=980	N=800	N=180	
Age	56.00 (48.00. 64.00)	55.00 (47.00. 64.00)	58.00 (50.00. 66.00)	**0.021**	58.00 (49.00. 65.00)	57.00 (49.00. 64.00)	61.00 (53.00. 67.00)	**0.002**
Sex (Female)	684 (61.18%)	531 (59.46%)	153 (68.00%)	**0.019**	612 (62.45%)	485 (60.62%)	127 (70.56%)	**0.013**
BMI	26.22 (5.06)	24.28 (3.10)	33.94 (3.83)	**<0.001**	26.13 (4.90)	24.37 (3.03)	33.94 (3.89)	**<0.001**
Current smokers Former smokers Never smokers	49 (4.42%) 324 (29.22%) 736 (66.37%)	37 (4.18%) 246 (27.80%) 602 (68.02%)	12 (5.36%) 78 (34.82%) 134 (59.82%)	0.068	32 (3.30%) 239 (24.66%) 698 (72.03%)	28 (3.53%) 201 (25.31%) 565 (71.16%)	4 (2.29%) 38 (21.71%) 133 (76.00%)	0.39
Onset asthma (age)	35.00 (20.00. 47.00)	34.00 (20.00. 46.00)	38.00 (20.00. 50.00)	0.11	33.00 (20.00. 45.00)	34.00 (20.00. 45.00)	30.00 (16.50. 45)	0.39
Diagnosis asthma (age) <18 (n. %) 18-39 (n. %) ≥40 (n. %)	172 (20.98) 300 (36.59) 348 (42.44)	141 (21.66) 243 (37.33) 267 (41.01)	31 (18.34) 57 (33.73) 81 (47.93)	0.261	186 (21.16) 354 (40.27) 339 (38.57)	146 (20.19) 302 (41.77) 275 (38.04)	40 (25.64) 52 (33.33) 64 (41.03)	0.113
Clinical status								
ACQ >1.5 or ACT <20 or uncontrolled according to GINA	690 (74.84%)	549 (74.09%)	141 (77.90%)	0.29	553 (56.43%)	446 (55.75%)	107 (59.44%)	0.37
ACT	15.06 (×/1.45)	15.36 (×/1.44)	13.94 (×/1.48)	**<0.001**	19.05 (×/1.35)	19.25 (×/1.34)	18.14 (×/1.38)	**0.025**
AQLQ	3.99 (×/1.40)	4.04 (×/1.40)	3.79 (×/1.37)	**0.032**	4.90 (×/1.37)	4.95 (×/1.37)	4.68 (×/1.37)	0.073

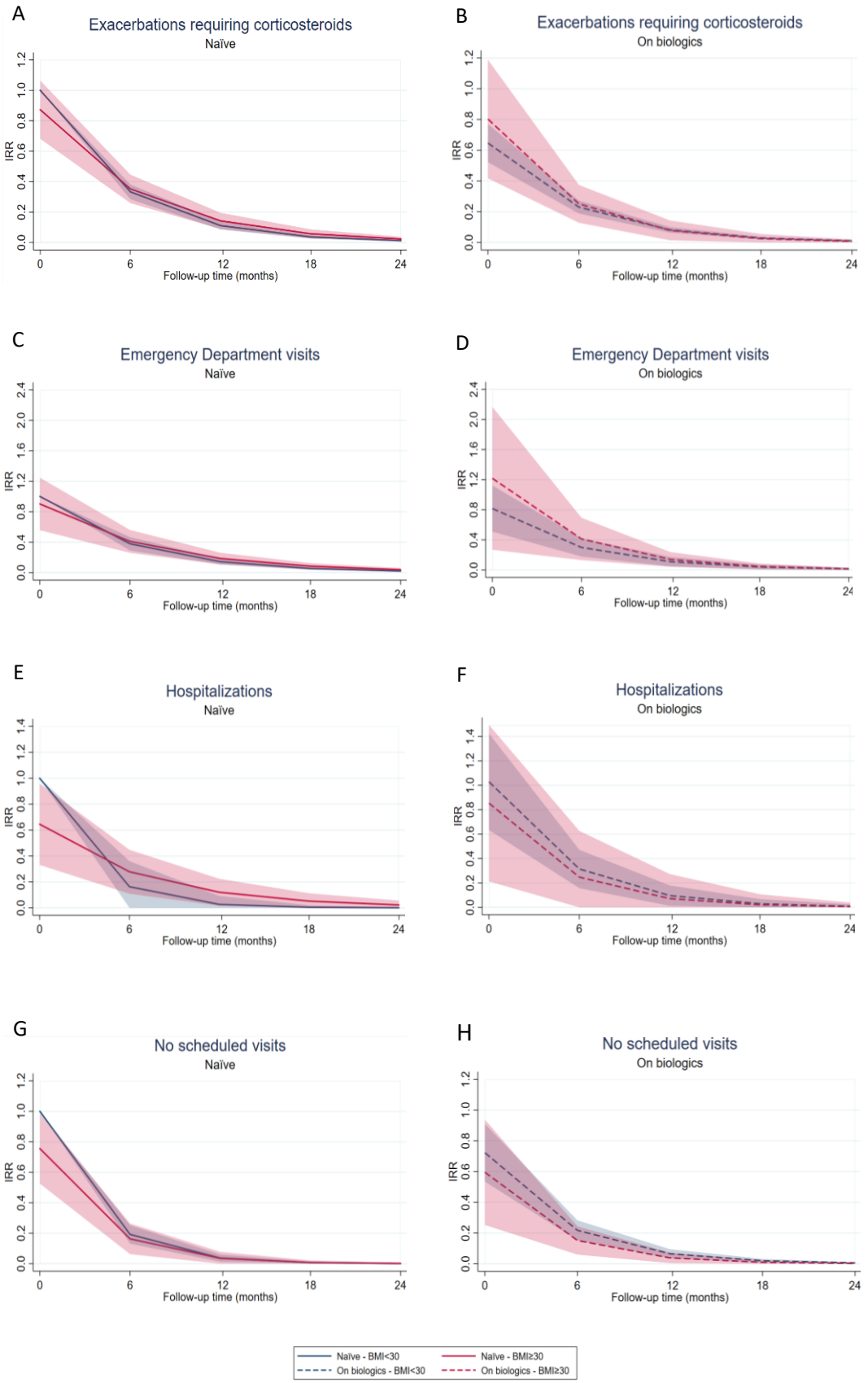
FEV1 post-bronchodilator <80% of predicted	284 (30.80%)	224 (30.23%)	60 (33.15%)	0.45	301 (30.71%)	250 (31.25%)	51 (28.33%)	0.44
FEV1 % pre-bronchodilator	74.00 (58.00. 89.00)	75.00 (58.00. 90.00)	71.00 (56.00. 83.00)	0.11	77.00 (65.00. 93.00)	77.00 (65.00. 93.00)	76.00 (66.00. 96.00)	0.83
FEV1 % post-bronchodilator	80.00 (66.00. 95.00)	80.00 (66.20. 96.00)	79.50 (60.00. 92.10)	~~0.32~~ 0.21	82.00 (69.30. 95.00)	82.00 (70.00. 95.00)	82.75 (66.00. 95.00)	0.72
Patients with ≥2 exacerbations treated with systemic CS >3 consecutive days in the year before biologic	516 (55.97%)	423 (57.09%)	93 (51.38%)	0.17	471 (48.06%)	401 (50.13%)	70 (38.89%)	**0.006**
Exacerbations requiring CS				0.78				0.25
0	275 (24.60%)	217 (24.30%)	58 (25.78%)		485 (49.49%)	405 (50.63%)	80 (44.44%)	
1	125 (11.18%)	104 (11.65%)	21 (9.33%)		131 (13.37%)	103 (12.88%)	28 (15.56%)	
2-5	517 (46.24%)	411 (46.02%)	106 (47.11%)		250 (25.51%)	205 (25.62%)	45 (25.00%)	
>5	201 (17.98%)	161 (18.03%)	40 (17.78%)		114 (11.63%)	87 (10.88%)	27 (15.00%)	
At least one hospitalization. ICU admission. or mechanical ventilation for asthma in the past year	64 (6.94%)	54 (7.29%)	10 (5.52%)	0.40	54 (5.51%)	46 (5.75%)	8 (4.44%)	0.49
Asthma related hospitalizations				0.57				0.19
0	914 (81.75%)	734 (82.19%)	180 (80.00%)		845 (86.22%)	694 (86.75%)	151 (83.89%)	
1	121 (10.82%)	93 (10.41%)	28 (12.44%)		63 (6.43%)	47 (5.88%)	16 (8.89%)	
2-5	34 (3.04%)	29 (3.25%)	5 (2.22%)		25 (2.55%)	23 (2.88%)	2 (1.11%)	
>5	49 (4.38%)	37 (4.14%)	12 (5.33%)		47 (4.80%)	36 (4.50%)	11 (6.11%)	
Asthma related ED visits				0.74				0.14
0	856 (76.57%)	690 (77.27%)	166 (73.78%)		808 (82.45%)	666 (83.25%)	142 (78.89%)	
1	113 (10.11%)	87 (9.74%)	26 (11.56%)		65 (6.63%)	50 (6.25%)	15 (8.33%)	
2-5	92 (8.23%)	72 (8.06%)	20 (8.89%)		40 (4.08%)	35 (4.38%)	5 (2.78%)	
>5	57 (5.10%)	44 (4.93%)	13 (5.78%)		67 (6.84%)	49 (6.13%)	18 (10.00%)	
No scheduled visits				0.80				**0.004**
0	555 (49.64%)	438 (49.05%)	117 (52.00%)		681 (69.49%)	568 (71.00%)	113 (62.78%)	
1	60 (5.37%)	47 (5.26%)	13 (5.78%)		44 (4.49%)	32 (4.00%)	12 (6.67%)	
2-5	252 (22.54%)	206 (23.07%)	46 (20.44%)		114 (11.63%)	98 (12.25%)	16 (8.89%)	
>5	251 (22.45%)	202 (22.62%)	49 (21.78%)		141 (14.39%)	102 (12.75%)	39 (21.67%)	
On treatment with: High doses of inhaled corticosteroids plus LABA or another controller	851 (92.30%)	685 (92.44%)	166 (91.71%)	0.74	935 (95.41%)	763 (95.38%)	172 (95.56%)	0.92
OCS for at least six months in the past year	296 (32.10%)	238 (32.12%)	58 (32.04%)	0.98	217 (22.14%)	181 (22.63%)	36 (20.00%)	0.44
Daily prednisone-equivalent dosage in the year before baseline	6.70 (5.00. 25.00)	6.70 (5.00. 25.00)	16.54 (5.00. 25.00)	0.68	5.00 (5.00. 25.00)	5.00 (5.00. 25.00)	16.00 (5.00. 25.00)	0.59
Biomarkers								
FeNO	34.03 (×/2.67)	37.32 (×/2.59)	23.53 (×/2.77)	**<0.001**	28.20 (×/2.54)	28.48 (×/2.56)	26.73 (×/2.48)	0.60
Eosinophils (n)	0.41 (0.20. 0.70)	0.43 (0.20. 0.71)	0.36 (0.14. 0.64)	**0.033**	0.20 (0.08. 0.50)	0.20 (0.08. 0.51)	0.23 (0.07. 0.49)	0.96
Neutrophils (n)	4.03 (3.12. 5.20)	3.90 (3.06. 5.14)	4.53 (3.53. 5.47)	**0.002**	3.90 (3.11. 5.12)	3.90 (3.11. 5.12)	4.07 (3.16. 5.14)	0.80
Total IgE	186.00 (80.70. 423.00)	188.00 (86.00. 421.00)	181.00 (62.50. 424.00)	0.33	227.00 (88.60. 538.00)	228.00 (85.00. 527.00)	211.80 (108.00.687.00)	0.43
Th2 profile				**0.023**				0.35
-low	516 (46.15%)	397 (44.46%)	119 (52.89%)		503 (51.33%)	405 (50.63%)	98 (54.44%)	
-high	602 (53.85%)	496 (55.54%)	106 (47.11%)		477 (48.67%)	395 (49.38%)	82 (45.56%)	
Comorbidities								

Allergy	644 (57.60%)	512 (57.33%)	132 (58.67%)	0.72	668 (68.16%)	545 (68.13%)	123 (68.33%)	0.96
Rhinitis				0.53				0.49
-never	412 (37.18%)	327 (36.91%)	85 (38.29%)		340 (35.20%)	273 (34.47%)	67 (38.51%)	
-yes. ongoing	578 (52.17%)	460 (51.92%)	118 (53.15%)		464 (48.03%)	382 (48.23%)	82 (47.13%)	
-yes. previous	118 (10.65%)	99 (11.17%)	19 (8.56%)		162 (16.77%)	137 (17.30%)	25 (14.37%)	
CRSsNP	309 (28.19%)	247 (28.23%)	62 (28.05%)	0.96	261 (27.13%)	226 (28.61%)	35 (20.35%)	**0.027**
CRSwNP	526 (47.13%)	449 (50.39%)	77 (34.22%)	**<0.001**	444 (45.77%)	377 (47.42%)	67 (38.29%)	**0.028**
OSAS	67 (6.37%)	30 (3.57%)	37 (17.45%)	**<0.001**	41 (4.39%)	21 (2.74%)	20 (11.90%)	**<0.001**
GI reflux	414 (37.67%)	315 (35.96%)	99 (44.39%)	**0.020**	369 (38.20%)	293 (36.99%)	76 (43.68%)	0.10
Cardiovascular disease	320 (30.19%)	211 (25.03%)	109 (50.23%)	**<0.001**	279 (30.53%)	196 (26.17%)	83 (50.30%)	**<0.001**
Type 2 diabetes	49 (4.62%)	29 (3.43%)	20 (9.30%)	**<0.001**	49 (5.34%)	27 (3.60%)	22 (13.02%)	**<0.001**
Osteoporosis	131 (13.60%)	103 (13.41%)	28 (14.36%)	0.73	135 (15.73%)	111 (15.68%)	24 (16.00%)	0.92
Mood disorders	107 (10.09%)	80 (9.47%)	27 (12.56%)	0.18	90 (9.83%)	72 (9.64%)	18 (10.65%)	0.69

Data are presented medians ± interquartile range (IQR), harmonic means (×/multiplicative SD) or number (%).
Abbreviations: BMI, body mass index; ACT, asthma control test; ACQ, asthma control questionnaire; GINA, Global Initiative for Asthma; AQLQ, asthma quality of life questionnaire; FEV1, forced expiratory volume in 1 second; CS, corticosteroid; ICU, intensive care unit; ED, emergency department; LABA, long-acting beta agonist; OCS, oral corticosteroids; FeNO, Fractional Exhaled Nitric Oxide; IgE, immunoglobulin E; CRSsNP, chronic rhinosinusitis without Nasal Polyps; CRSwNP, chronic rhinosinusitis with Nasal Polyps; OSAS, obstructive sleep apnoea syndrome; GI, gastrointestinal.

Over 24 months, zero-inflated negative binomial (ZINB) models, adjusted for relevant covariates (age, sex, ACT, FEV1, blood eosinophils, CRwNP, and treatment duration for on-treatment patients), showed no clinically meaningful differences between obese and non-obese patients in exacerbations requiring corticosteroids, asthma-related emergency visits, hospitalizations, or unscheduled visits (Figure 8; Table 3). These findings suggest that outcome rates declined substantially over time, irrespective of BMI, in both biologic-naïve and on-treatment cohorts.

Figure 8. Predicted rate of asthma exacerbations over time by treatment group and BMI category.



Longitudinal estimates of exacerbation rates (events/year) are shown separately for biologic-naïve patients (A, C, E, G) and those already receiving biologics (B, D, F, H), stratified by BMI category (<30 vs ≥30). Models were fitted using zero-inflated negative binomial (ZINB) regression and adjusted for baseline age, sex, presence of chronic rhinosinusitis with nasal polyps (CRSwNP), blood eosinophil count, FEV_1 (% predicted), ACT score, and number of days on biologic therapy at baseline. The reference group consists of naïve patients with BMI <30 at baseline. Shaded areas represent 95% confidence intervals. IRR, incidence rate ratio.

Table 3. ZINB-estimated IRR for exacerbations (A), emergency department visits (B), hospitalizations (C), and unscheduled visits (D), stratified by BMI and treatment group.

A. Exacerbations requiring systemic corticosteroid

	Naïve				On biologics			
Time (months)	**BMI<30**		**BMI≥30**		**BMI<30**		**BMI≥30**	
	n	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**
0	665	Reference	166	0.87 (0.68; 1.06)	591	0.65 (0.52; 0.77)	133	0.80 (0.42; 1.19)
6	607	0.33 (0.28; 0.38)	143	0.35 (0.26; 0.45)	547	0.23 (0.19; 0.27)	119	0.25 (0.13; 0.37)
12	488	0.11 (0.09; 0.14)	117	0.14 (0.09; 0.20)	439	0.08 (0.06; 0.10)	92	0.08 (0.01; 0.14)
18	404	0.04 (0.03; 0.05)	94	0.06 (0.03; 0.09)	369	0.03 (0.02; 0.04)	79	0.03 (0.00; 0.06)
24	335	0.01 (0.01; 0.02)	81	0.02 (0.01; 0.04)	314	0.01 (0.01; 0.01)	70	0.01 (0.00; 0.02)

Multi-adjusted for baseline age, sex, CRSwNP*, baseline eosinophils (n), baseline FEV1, baseline ACT, days on biologic therapy at baseline.*

B. Emergency Department visits

	Naïve				On biologics			
Time (months)	**BMI<30**		**BMI≥30**		**BMI<30**		**BMI≥30**	
	n	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**
0	681	Reference	169	0.90 (0.56; 1.25)	588	0.82 (0.51; 1.12)	130	1.22 (0.27; 2.17)
6	609	0.38 (0.29; 0.47)	144	0.41 (0.26; 0.56)	552	0.30 (0.18; 0.42)	121	0.41 (0.13; 0.69)
12	489	0.14 (0.10; 0.18)	117	0.19 (0.11; 0.26)	440	0.11 (0.05; 0.17)	92	0.14 (0.04; 0.24)
18	405	0.05 (0.04; 0.07)	94	0.08 (0.04; 0.12)	370	0.04 (0.01; 0.07)	79	0.05 (0.01; 0.09)
24	337	0.02 (0.01; 0.03)	81	0.04 (0.02; 0.06)	314	0.01 (0.00; 0.03)	70	0.02 (0.00; 0.03)

Multi-adjusted for baseline age, sex, CRSwNP, baseline eosinophils (n), baseline FEV1, baseline ACT, days on biologic therapy at baseline.

C. Hospitalizations

	Naïve				On biologics			
Time (months)	**BMI<30**		**BMI≥30**		**BMI<30**		**BMI≥30**	
	n	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**

0	686	Reference	170	0.65 (0.33; 0.96)	600	1.03 (0.63; 1.42)	137	0.85 (0.21; 1.49)
6	609	0.16 (0.00; 0.36)	143	0.28 (0.11; 0.45)	552	0.31 (0.16; 0.47)	121	0.25 (0.00; 0.63)
12	489	0.03 (0.00; 0.09)	117	0.12 (0.02; 0.22)	440	0.10 (0.01; 0.18)	92	0.07 (0.00; 0.27)
18	405	0.00 (0.00; 0.02)	94	0.05 (0.00; 0.11)	370	0.03 (0.00; 0.07)	79	0.02 (0.00; 0.11)
24	337	0.00 (0.00; 0.00)	81	0.02 (0.00; 0.06)	314	0.01 (0.00; 0.02)	70	0.01 (0.00; 0.04)

Multi-adjusted for baseline age, sex, CRSwNP, baseline eosinophils (n), baseline FEV1, baseline ACT, days on biologic therapy at baseline.

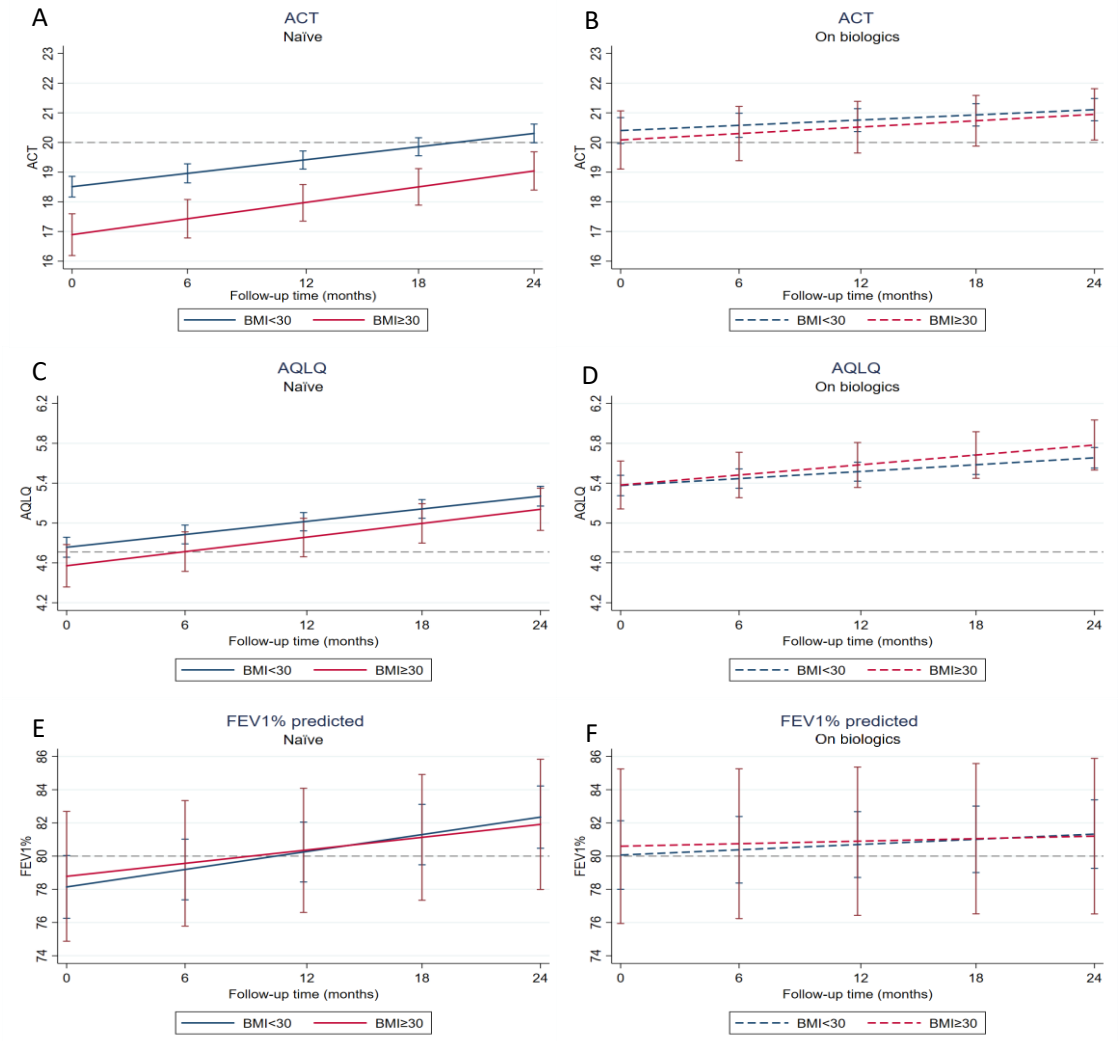
D. Unscheduled visits

	Naïve				**On biologics**			
Time (months)	**BMI<30**		**BMI≥30**		**BMI<30**		**BMI≥30**	
	n	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**	**n**	**IRR (95% CI)**
0	556	Reference	140	0.76 (0.53; 0.99)	547	0.72 (0.54; 0.91)	113	0.60 (0.25; 0.94)
6	519	0.19 (0.13; 0.25)	134	0.16 (0.06; 0.26)	540	0.22 (0.15; 0.28)	104	0.15 (0.06; 0.24)
12	453	0.04 (0.01; 0.06)	108	0.04 (0.00; 0.08)	432	0.07 (0.04; 0.10)	88	0.04 (0.01; 0.07)
18	378	0.01 (0.00; 0.01)	87	0.01 (0.00; 0.02)	366	0.02 (0.01; 0.03)	77	0.01 (0.00; 0.02)
24	314	0.00 (0.00; 0.00)	78	0.00 (0.00; 0.01)	308	0.01 (0.00; 0.01)	69	0.00 (0.00; 0.01)

Multi-adjusted for baseline age, sex, CRSwNP, baseline eosinophils (n), baseline FEV1, baseline ACT, days on biologic therapy at baseline.
Zero-inflated negative binomial (ZINB) regression models estimating incidence rate ratios (IRR) for exacerbations requiring systemic corticosteroids, emergency department visits, hospitalizations, and unscheduled visits. Results are stratified by BMI category (<30 vs. ≥30), treatment status (biologic-naïve vs. on biologics), and follow-up time (0–24 months). Models are adjusted for baseline age, sex, CRSwNP, eosinophils, FEV1, ACT, and days on biologic therapy at inclusion. The reference category is the biologic-naïve group at baseline (T=0), which allows IRR comparisons both within the naïve population over time and between naïve and on-treatment patients.

Linear mixed-effects models estimating longitudinal changes in ACT, AQLQ, and FEV1% predicted confirmed similar trajectories across BMI categories in both groups (naïve vs on-treatment). In biologic-naïve patients, ACT scores were consistently lower in obese individuals, though the difference did not reach the threshold of clinical relevance (≥3 points). AQLQ and FEV1% showed overlapping confidence intervals, with no consistent differential pattern by BMI. In on-treatment patients, trajectories remained relatively flat and within a narrow range, regardless of BMI, with higher baseline values reflecting previous treatment benefits (Figure 9).

Figure 9. Predicted rate of ACT, AQLQ and FEV_1% improvement over time by treatment group and BMI category.



Linear mixed-effects models were used to estimate changes in Asthma Control Test (ACT) scores, Asthma Quality of Life Questionnaire (AQLQ) scores, and percent predicted FEV_1 over 12 months of follow-up. Analyses were stratified by BMI category (<30 vs ≥30) and conducted separately for biologic-naïve patients (A, C, E) and those receiving biologics (B, D, F). Models were adjusted for baseline age, sex, presence of chronic rhinosinusitis with nasal polyps (CRSwNP), baseline blood eosinophil count, baseline ACT score, number of exacerbations in the previous year, and number of days on biologic therapy at baseline. Error bars represent 95% confidence intervals.

Sensitivity analyses for ZINB and mixed-effects models with BMI as a continuous variable yielded consistent results, with no differential trajectories observed across BMI strata for exacerbations, healthcare utilization, ACT, AQLQ, or FEV1.

Kaplan–Meier analyses restricted to patients not in remission at baseline estimated the time to partial or complete remission. At enrollment, partial or complete remission was observed in 20,4% of

biologic-naïve and 46,7% of on-treatment patients, with no BMI-related differences. These patients were excluded from survival analysis (Table 4).

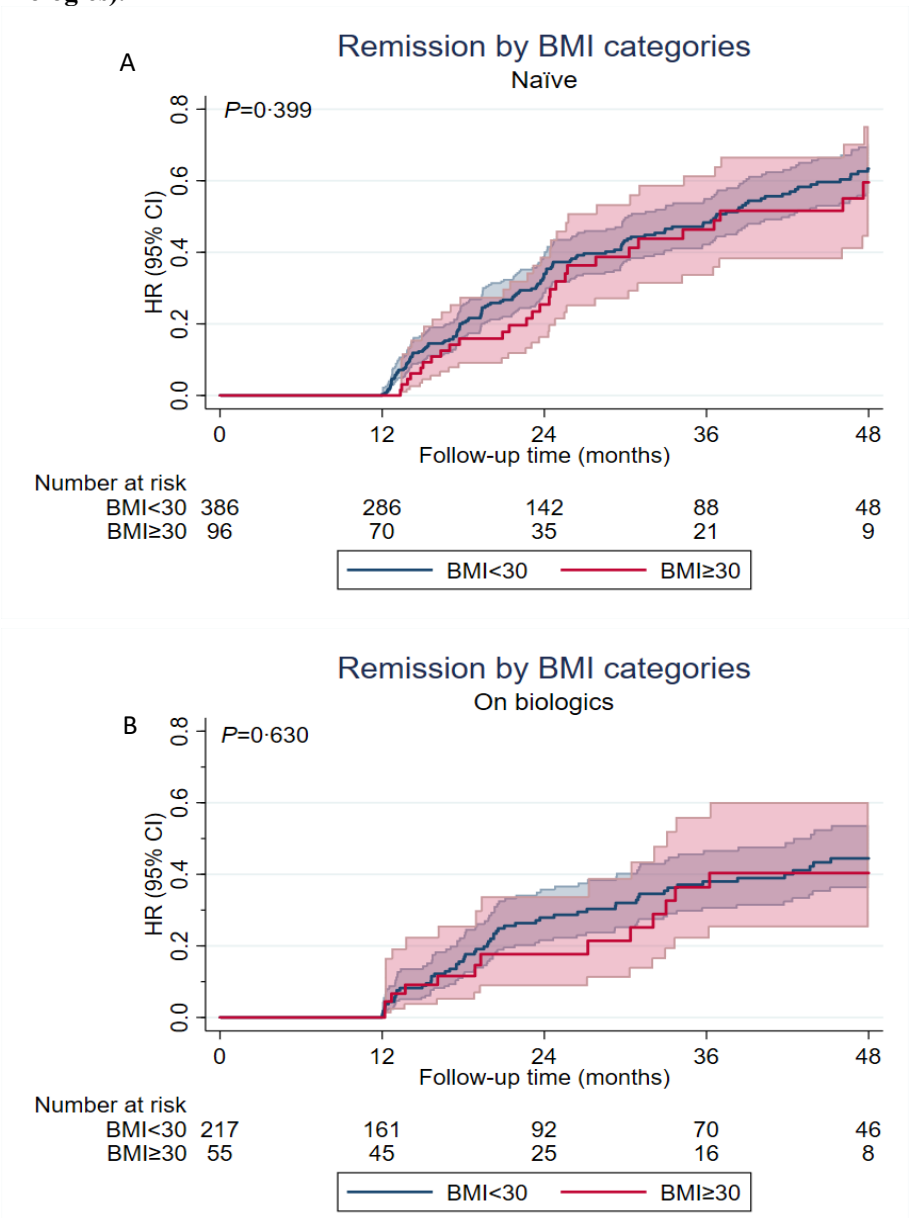
Table 4. Baseline Clinical Remission Status According to BMI and Biologic Exposure

	Biologic-naïve at inclusion				**On biologics at inclusion**			
	Total	**BMI<30**	**BMI≥30**	**P**	**Total**	**BMI<30**	**BMI≥30**	***P***
	N=711	N=565	N=146		N=599	N=494	N=105	
Clinical remission				0.612				0.203
No remission	566	450 (79.65 %)	116 (79.45%)		319	255 (51.62%)	64 (60.95%)	
Partial remission	122	95 (16.81%)	27 (18.49%)		176	149 (30.16%)	27 (25.71%)	
Complete remission	23	20 (3.54%)	3 (2.05%)		104	90 (18.22%)	14 (13.33%)	

Baseline clinical remission status, defined according to SANI criteria. Frequencies of no remission, partial remission, and complete remission are reported for biologic-naïve and on-biologic patients, stratified by BMI category.

Kaplan–Meier curves showed no significant difference in time to partial or complete remission between obese and non-obese patients in either cohort (biologic-naïve or on-treatment) (Figure 10). At 24 months, the cumulative probability of remission was 22-25% across BMI strata, with overlapping 95% confidence intervals. The early plateau observed in the first 12 months reflected the requirement for 12 consecutive months fulfilling remission criteria and possible early dropouts. Dropout rates and adverse events did not differ by BMI, excluding differential attrition as a source of bias. Follow-up time did not differ across BMI categories in either group (naïve 671,5 vs 651 days, p=0,151; on-treatment 761 vs 718 days, p=0,439).

Figure 10. Complete or partial remission stratified by BMI and treatment status (Naïve vs. On Biologics).



Kaplan–Meier curves show the probability of achieving partial or complete clinical remission over time among patients stratified by BMI category (<30 vs ≥30) and biologic treatment status (naïve [a] vs on biologics [b]). The analysis included only patients who had not achieved remission at baseline. Shaded areas indicate 95% confidence intervals. HR, hazard ratio.

Multivariable Cox regression models, adjusted for age, sex, ACT, FEV1, blood eosinophils, CRwNP, and baseline exacerbations (plus treatment duration for on-treatment patients), indicated that obesity did not influence the likelihood of remission (naïve: HR 0,88 [95% CI 0·57–1·37]; p=0·806; on-treatment: HR 0,91 [0·44–1·89]; p=0·320; Table 5). Similar results were obtained when modelling BMI as a continuous variable (naïve: HR 0,99 [95% CI 0·95–1·02]; p=0·450; on-treatment: HR 0,95

[0·89–1·01]; p=0·104; Table 5), and by sensitivity analysis accounting for centre-level variability using a shared frailty term. Two further sensitivity analyses supported the robustness of the findings: (1) excluding the 71 patients who initiated biologic therapy within one month before enrolment did not change the estimates, and (2) starting the risk period at 12 months (when remission can first be observed by definition) produced similar results, indicating no immortal time bias. Another sensitivity analysis modelling BMI as a continuous variable using restricted cubic splines showed no evidence of non-linear associations between BMI and remission, with spline-derived estimates remaining close to unity. Consistent findings were also obtained when patients were stratified by BMI categories (<25, 25–29, ≥30), showing a non-significant trend toward better outcomes in patients with normal weight compared to those who were overweight or obese, particularly among patients already on biologic therapy. A separate sensitivity analysis stratifying patients by smoking status showed no significant differences in remission trajectories across smoking categories.

Finally, to assess the potential influence of missing data on our results, multiple imputation was conducted as an additional sensitivity analysis. The imputed models yielded effect estimates consistent with the complete-case analyses, showing no influence of obesity on the likelihood of remission, thereby confirming the robustness of the original findings with a larger analytic sample.

Table 5. Cox models estimated Hazard Ratios of incident partial or complete remission, stratified by treatment group.

	Naive		**On biologics**	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
BMI≥30	0.88 (0.57, 1.37)	*0.586*	0.91 (0.44, 1.89)	*0.806*
Age	1.00 (0.98, 1.01)	*0.686*	1.00 (0.97, 1.02)	*0.794*
Sex (Female)	0.98 (0.69, 1.39)	*0.929*	0.70 (0.41, 1.18)	*0.184*
CRwNP	0.94 (0.68, 1.31)	*0.736*	1.26 (0.68, 1.89)	*0.653*
BEC	1.02 (0.99, 1.05)	*0.111*	0.78 (0.41, 1.49)	*0.453*
FEV_1% (pre-bronchodilator)	1.00 (1.00, 1.01)	***0.046***	1.00 (0.99, 1.01)	*0.635*
ACT	0.99 (0.96, 1.03)	*0.719*	1.04 (0.99, 1.10)	*0.127*
Exacerbations	0.99 (0.92, 1.05)	*0.681*	1.00 (0.85, 1.16)	*1.000*
Days on biologic therapy	-	-	1.00 (0.99, 1.00)	*0.134*

	Naive		On biologics	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
BMI	0.99 (0.95, 1.02)	*0.450*	0.95 (0.89, 1.01)	*0.104*

BMI was analysed both as a categorical variable (≥30 vs <30 kg/m²) and as a continuous variable (results shown in the lower panel of the table).
BMI, body mass index; CRwNP, chronic rhinosinusitis with nasal polyps; BEC, blood eosinophil count; FEV_1%, forced expiratory volume at 1 second.

3.5 Discussion

In this large real-world analysis of nearly 2,100 patients from the SANI registry, obesity, although associated with a distinct clinical and inflammatory profile, did not significantly influence the long-term effectiveness of biologic therapy in severe asthma. Over time, both obese and non-obese patients demonstrated sustained and comparable improvements across all key outcomes, including reductions in exacerbations, improved ACT and AQLQ scores, and enhanced lung function as reflected by FEV_1. Although ACT scores remained marginally lower in biologic-naïve obese patients, the difference was clinically negligible, and symptom trajectories were largely parallel between groups [62]. Remission accrued progressively in both obese and non-obese patients, frequently becoming evident only after two or more years of continuous treatment, underscoring the importance of prolonged follow-up to fully capture the therapeutic benefits of biologic agents. Importantly, these findings were robust across multiple sensitivity analyses, including modelling BMI as a continuous variable (both linearly and using restricted cubic splines), stratification into three BMI categories, stratification by smoking status, and adjustment for centre-level clustering.

These findings are particularly noteworthy in light of the baseline disadvantage observed among obese patients, who presented with poorer asthma control, reduced quality of life, and a higher burden of comorbidities, including OSAS, GERD, cardiovascular disease, and type 2 diabetes. Such conditions complicate disease management, restrict therapeutic options, and are generally associated with poorer outcomes [63]–[65]. Similarly, baseline differences in type 2 (T2) inflammatory biomarkers did not translate into differential treatment responses. Obese patients, although belonging to a population with

high T2 inflammation and eligibility for biologic therapy, exhibited a relatively attenuated T2-high signature, a lower prevalence of CRwNP, and higher neutrophil counts. These features were particularly evident in the biologic-naïve cohort, where biomarker levels were unaffected by prior treatment. Nevertheless, this attenuation of T2 inflammation was insufficient to compromise the effectiveness of targeted biologic therapies. It may, however, help explain the slightly lower proportion of obese patients experiencing two or more exacerbations, a trend that reached statistical significance only in the on-treatment cohort, as a blunted T2 profile could plausibly reduce susceptibility to acute exacerbations once adequate disease control is achieved.

Our findings contrast with those of some registry-based studies, such as the Danish Severe Asthma Register (DSAR) and the UK Severe Asthma Registry (UKSAR), which identified obesity as a negative predictor of remission at annual review [53],[56]. Subsequent analyses from the UKSAR refined this observation, demonstrating that biologics were effective across all BMI categories and that the attenuated responses observed in severely obese patients were largely attributable to their worse baseline clinical profile; however, those analyses focused on outcomes such as exacerbations and asthma control rather than remission [66]. A recent meta-analysis of observational studies suggested a significant negative effect of obesity on remission, albeit with substantial heterogeneity in outcome definitions and observation windows. By contrast, the International Severe Asthma Registry (ISAR) did not identify obesity as a predictor of treatment response [58].

A key methodological distinction between our analysis and previous observational or registry-based studies lies in our stricter and temporally consistent definition of remission. We required all remission criteria, absence of OCS use, no exacerbations, controlled symptoms, and pre-bronchodilator FEV_1 ≥80% predicted, to be fulfilled at each assessment over 12 consecutive months. In contrast, in UKSAR, remission is assessed at annual review visits that may occur between 9 and 24 months after the previous evaluation. This variability introduces substantial heterogeneity: intervals shorter than 12 months may fail to demonstrate sustained disease control, whereas longer intervals may overestimate stability by implicitly assuming continuous control throughout the period. In ISAR, exacerbations are reported as annualized rates derived from events occurring within a variable follow-up window of 48–80 weeks after biologic initiation, while other remission domains, ACT, FEV_1, and OCS use, are

captured at the visit closest to one year, provided it falls within a broad time window. Both approaches differ fundamentally from our requirement for concurrent and sustained fulfilment of all remission domains over 12 consecutive months. Variability in remission definitions was also evident in the studies included in the meta-analysis by Shackleford et al., which identified up to 48 different definitions of clinical remission across studies [56]. For these reasons, remission estimates at 24 months in our analysis are not directly comparable with those reported elsewhere [53],[56]–[58]. Using time-to-event methods, we observed cumulative remission rates of approximately 22–25% by 24 months, acknowledging that remission could not, by definition, be ascertained before 12 months. Although numerically similar to rates reported in other registries, those estimates were derived using variable assessment schedules.

Conversely, our analysis encompassed both partial and complete remission, which may further contribute to heterogeneity and may partially explain the higher estimates compared with studies applying either definition alone. The lung function component of remission also warrants specific consideration. Whereas some definitions require stability of FEV_1 over time and others mandate a threshold value, we adopted a pre-bronchodilator FEV_1 ≥80% predicted, corresponding to normal lung function. Over observation periods extending up to four years, defining stability solely in terms of absolute or relative changes in FEV_1 is challenging, particularly in light of expected age-related decline. Requiring sustained normal lung function was therefore considered methodologically robust, although this more stringent criterion may have resulted in conservative remission estimates. Further studies are needed to establish evidence-based criteria for lung function remission, including whether age-adjusted reference equations are sufficient or whether additional measures are required [67].

We were unable to assess whether weight reduction during follow-up, either through OCS tapering or lifestyle modification, contributed to the observed treatment responses [68]. This represents an important area for future research, as emerging evidence suggests that intentional weight loss may improve asthma control, reduce systemic inflammation, and potentially enhance biologic efficacy. In addition, weight reduction may mitigate obesity-related comorbidities such as OSAS and GERD, thereby attenuating their negative impact on asthma management.

Several limitations should be acknowledged. The observational nature of the registry precludes causal inference. BMI was the sole measure of obesity, limiting the ability to distinguish between different obesity phenotypes or to account for body composition and metabolic status. Information on early-life weight trajectories and on the distinction between steroid-induced and primary obesity was unavailable, precluding exploration of potentially distinct pathophysiological mechanisms influencing treatment response. Although peripheral biomarkers such as blood eosinophils and FeNO were available, more detailed assessments of airway inflammation were lacking. The remission analysis was based on a smaller sample and may have been underpowered to detect subtle between-group differences; however, effect estimates were consistently close to unity with overlapping confidence intervals, suggesting that any undetected differences are unlikely to materially affect remission trajectories. Sensitivity analyses using multiple yielded results fully consistent with those of the primary model, supporting the robustness of our findings. A total of 94 patients who had discontinued biologic therapy before baseline were excluded, in line with our focus on longitudinal outcomes under ongoing treatment. Although this exclusion may have introduced attrition-related bias, the proportions of obese and non-obese individuals among early discontinuers closely mirrored those observed in the on-treatment cohort. In addition, 129 registered patients were excluded due to missing baseline BMI, which may have reduced statistical power and introduced bias, as non-random recording of BMI in routine clinical practice cannot be excluded. Among biologic-naïve patients, a modest and non-significant increase in 12-month treatment discontinuation was observed in those with BMI ≥30 kg/m² (33% vs 29%), together with a slightly shorter follow-up (~1% difference), which does not allow definitive exclusion of differential attrition despite comparable dropout proportions and reasons across BMI categories. Only one death was recorded during follow-up. Although literature-based mortality estimates for severe asthma, largely derived from the pre-biologic era, would suggest higher expected rates, the marked reductions in exacerbations, hospitalisations, and emergency visits observed after biologic initiation, consistent with previous biologic studies, indicate that mortality in this population is likely substantially lower than in historical cohorts. Therefore, while some underestimation cannot be ruled out, a meaningful competing-risk effect is unlikely to have materially influenced the survival

analyses. Finally, the relatively small number of patients treated with dupilumab or tezepelumab limits the strength of conclusions for these specific agents.

Despite these limitations, the real-world setting, heterogeneous population, multicentre design, and longitudinal follow-up substantially strengthen the generalisability of our findings. Outcomes were assessed repeatedly over a follow-up period of up to four years, allowing the capture of both delayed and sustained remission dynamics and providing a more nuanced depiction of long-term disease control than previously reported [53],[56]–[58]. Comparative analyses between obese and non-obese patients were remarkably consistent across all major endpoints, including ACT, AQLQ, FEV_1, exacerbation rates, and remission, reinforcing the internal coherence and robustness of the results. Extensive sensitivity analyses, encompassing multiple BMI modelling strategies and adjustment for centre-level clustering, confirmed that findings were not driven by modelling assumptions or site-specific effects. Data were derived from more than 60 specialised asthma centres across Italy, ensuring broad geographic and clinical representation, with no evidence of meaningful between-centre variability.

Taken together, these findings provide strong real-world evidence that biologic therapies remain effective in obese patients with type 2–high severe asthma, irrespective of baseline BMI. Key clinical outcomes, including exacerbation frequency, asthma control, lung function, and remission, improved consistently over time across BMI categories. Adjunct weight-management strategies, including dietary modification, structured physical activity, and, when appropriate, pharmacological interventions such as GLP-1 receptor agonists, may further enhance remission rates. Future studies should move beyond BMI-based classifications to investigate whether distinct obesity phenotypes and metabolic–inflammatory profiles influence biologic responsiveness and could support more refined, personalised treatment strategies.

4. STUDY 2 - MULTIDOMAIN PROFILING FOR DIAGNOSTIC DISCRIMINATION OF COGNITIVE DECLINE IN GERIATRIC OUTPATIENTS

4.1 Background

Cognitive decline refers to a deterioration in cognitive performance relative to an individual's previous level and age-expected norms. It becomes clinically relevant when it is persistent, exceeds what would be anticipated for healthy aging, affects real-world performance, or signals an underlying neurodegenerative or cerebrovascular process. Importantly, cognitive decline spans a continuum, from subtle, subjective changes to objective impairment measurable on neuropsychological testing, and ultimately to overt dementia. Along this continuum, the clinical expression of brain pathology is highly heterogeneous and is shaped by interindividual differences in cognitive reserve and compensatory capacity [69].

Within the broader spectrum of cognitive decline, dementia represents the stage at which impairment is no longer confined to test performance but manifests as a sustained loss of functional independence. Dementia is therefore defined not simply by deficits across cognitive domains such as memory, executive functions, attention, language, and visuospatial abilities but by the accompanying decline in everyday functioning that compromises autonomy and progressively increases care needs [70],[71]. This functional deterioration, affecting instrumental and eventually basic activities of daily living, is the hallmark that distinguishes dementia from earlier stages of impairment and constitutes a principal driver of individual burden and healthcare utilization.

A key point in geriatric cognitive evaluation is that physiological brain aging and dementia should be regarded as distinct processes rather than sequential points along a single linear trajectory. Normal brain senescence is typically associated with mild, gradual structural and functional changes such as modest reductions in brain volume, decreased synaptic plasticity, and slower information processing while functional autonomy is largely preserved. By contrast, dementia reflects a pathological state in which neurodegenerative and/or vascular damage exceeds compensatory capacity, resulting in irreversible synaptic and neuronal loss and, frequently, abnormal protein aggregation and neurovascular dysfunction [72].

Dementia is best understood as a syndrome rather than a single disease entity, referring to a clinical state in which cognitive impairment is accompanied by functional consequences and can arise from multiple underlying neuropathologies that frequently coexist in older adults [69],[70]. Alzheimer's disease (AD) is the most common cause, accounting for an estimated 60%–80% of cases, but other major etiologies include vascular dementia (approximately 5%–10%), dementia with Lewy bodies (around 5%, likely underdiagnosed in routine practice), and frontotemporal degeneration (about 3% of cases with onset at age ≥65 years and ~10% with onset before 65 years) [70]. Importantly, these categories are not mutually exclusive: more than half of individuals diagnosed clinically with AD may have "mixed dementia," with more than one identifiable contributing pathology often combining neurodegenerative and vascular mechanisms helping to explain heterogeneity in clinical presentation and progression [70],[73]. In both clinical and research contexts, dementia is nevertheless often discussed as though it were synonymous with AD, reflecting AD's predominance and the centrality of amyloid and tau frameworks in contemporary biomarker-driven research; however, this shorthand is conceptually imprecise and can obscure the high frequency of mixed and non-AD pathologies, particularly in geriatric populations where multimorbidity and vascular burden are common.

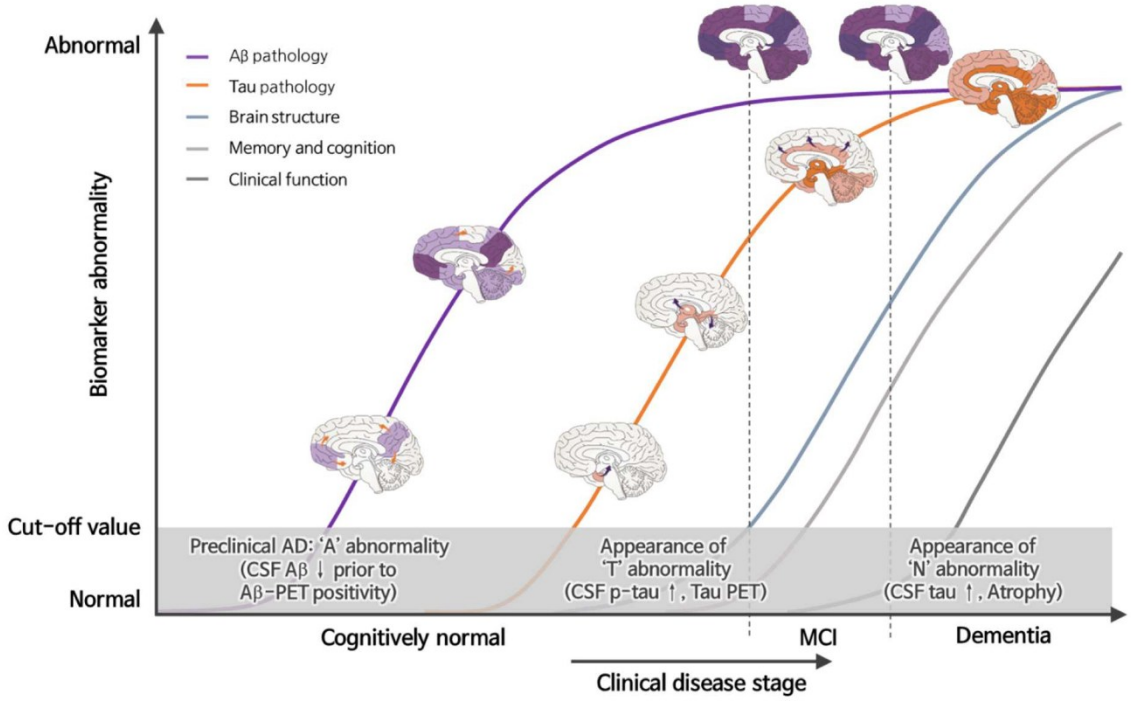
4.1.1 *Continuum model of cognitive decline*

A major shift in how cognitive impairment is understood is the recognition that neurodegenerative diseases, most notably Alzheimer's disease (AD), develop over a prolonged continuum, with biological changes accumulating for years before dementia becomes clinically apparent. This perspective positions dementia as a late clinical expression of an evolving pathophysiological process and underpins efforts toward earlier identification, more refined prognostic stratification, and timely preventive or disease-modifying strategies [69],[74]. Importantly, progression is not uniform: symptom profiles and rates of decline vary widely, particularly in older adults in whom multimorbidity, vascular burden, and mixed neuropathologies are common. Neuropathologically, AD is defined by amyloid-β (Aβ) plaques, hyperphosphorylated tau neurofibrillary tangles, and downstream neurodegeneration [69],[73]. These abnormalities can be detected well before diagnosis: pathological changes may begin approximately 15–20 years prior to clear cognitive symptoms, with Aβ often accumulating one to two

decades before clinical decline and tau-related alterations emerging years before overt dementia [75]. Such timing supports the concept that clinical impairment reflects the downstream consequences of long-standing synaptic and neuronal dysfunction leading to progressive brain atrophy.

Clinically, the continuum is commonly described in broad stages. Preclinical phase features evidence of AD pathology without overt impairment and includes presymptomatic genetic forms (APP/PSEN1/PSEN2 mutations or trisomy 21) as well as biomarker-positive, cognitively normal individuals in the general population, an appreciable proportion among older adults (≈20–40%) [69]. With further progression, some individuals report subjective cognitive decline, often framed as subjective memory complaints, which may precede objective deficits and can be modulated by mood, sleep, and general health [73]. The subsequent prodromal phase is typically operationalized as mild cognitive impairment (MCI), conceived not as a single "memory disorder" but as a heterogeneous clinical category comprising distinct subtypes with different neurocognitive profiles and potential trajectories most notably amnestic MCI, which is more often associated with progression to AD; multidomain MCI, involving impairments across multiple domains (with or without memory involvement) and at risk of progression to AD or other dementias; and single-domain non-amnestic MCI, affecting a non-memory domain (e.g., language, executive, or visuospatial functions) and more frequently linked to non-AD dementias, including frontotemporal, Lewy body, and vascular forms[76]. Finally, AD dementia is characterized by more widespread cognitive deficits accompanied by clinically significant functional impairment (Figure 11).

Figure 11. Biomarkers and changes in spatiotemporal across the continuum of cognitive decline [75].



Acronyms: CSF, Cerebrospinal Fluid; PET, Positron Emission Tomography

To support etiologic attribution and staging across this spectrum, contemporary frameworks increasingly incorporate biomarker-based models such as AT(N)/ATN, integrating measures of amyloid, tau, and neurodegeneration obtained from CSF, PET, and, increasingly, blood-based assays while maintaining clinical phenotype as the cornerstone for diagnosis and care planning [73]. Within this integrated framework, subjective memory complaints and MCI represent pivotal transitional clinical states and motivate a more detailed discussion of MCI phenotypes and trajectories.

4.1.2 *Burden of dementia*

Accordingly, in geriatric medicine and memory-clinic practice, dementia is best conceptualized as a *syndrome* a final common clinical pathway arising from heterogeneous and often coexisting brain pathologies rather than a single disease entity. This syndromic nature drives substantial variability in presentation and progression. It also contributes to dementia's major public health impact, given its association with sustained cognitive and functional decline, frequent neuropsychiatric symptoms and frailty, and increased vulnerability to adverse health events, all of which translate into escalating health

and social care needs [69],[70]. For patients, it compromises complex daily activities and quality of life; for caregivers, it generates substantial long-term psychological and practical burden, often intensified by behavioural symptoms [77],[78]. At the societal level, the combination of long disease duration and population aging drives rising costs and service demand, reinforcing the need for life-course, equitable prevention and care strategies [79].

4.1.3 *Epidemiology of cognitive decline*

Dementia is largely a condition of later life, with prevalence increasing steeply with age; consequently, its population burden is highly sensitive to demographic aging. Worldwide, more than 55 million people live with dementia and nearly 10 million new cases occur each year, with over 60% of cases in low- and middle-income countries settings where limited diagnostic and care resources can widen inequities. Prevalence rises from roughly 3% at ages 65–70 to about 35% at ages ≥90, a gradient that is particularly relevant for geriatric outpatient services [79]. In Italy, one of the world's oldest populations, nearly one quarter of citizens were ≥65 years in 2025 (≈14.5 million), and adults ≥65 may exceed one third of the population by 2050; national estimates suggest about 1.2 million people currently live with dementia [80].

The current data and future projections highlight dementia as a significant and growing public health challenge, with profound implications for both the health systems and economies of countries worldwide.

4.1.4 *Pathophysiology of cognitive decline*

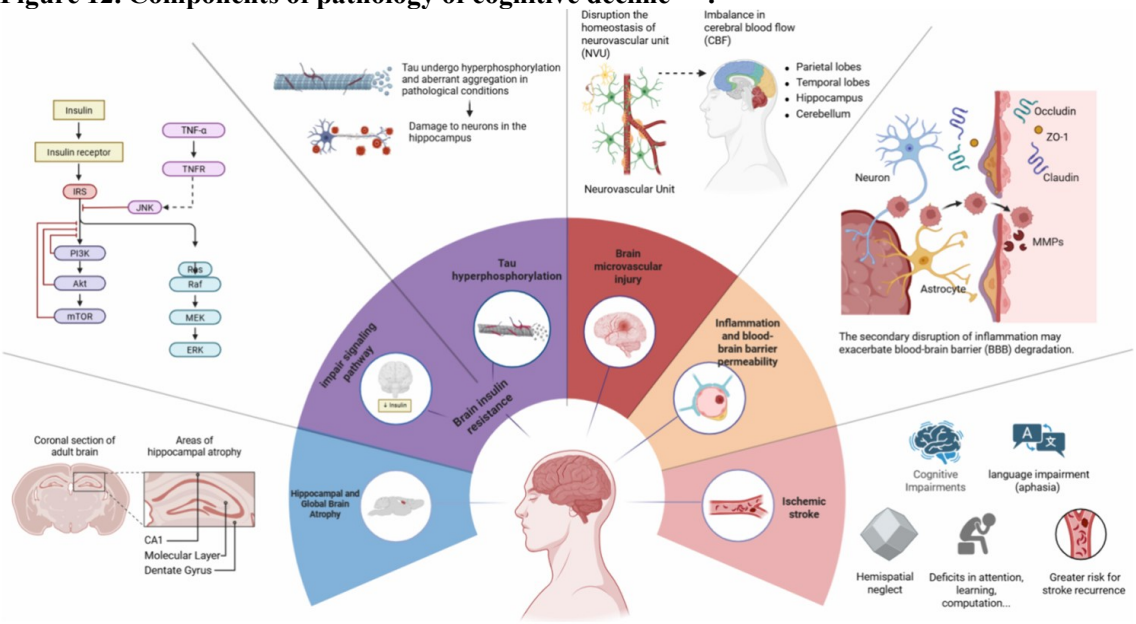
Brain aging and cognitive decline

Brain aging is a multifactorial biological process driven by a combination of molecular, cellular, and systemic changes that progressively impair the brain's ability to maintain homeostasis and adapt to stressors. Over time, these changes reduce neuronal plasticity and compromise synaptic function, which, when compounded by disease processes, sets the stage for cognitive decline. This progressive vulnerability reflects an imbalance between injury and compensation, with a gradual reduction in the

brain's capacity to repair and protect its structures. As a result, cognitive decline becomes more likely when the brain's compensatory mechanisms are overwhelmed by damage accumulation.

While brain aging is typically marked by mild, gradual structural and functional changes such as a reduction in brain volume and slowed information processing pathological cognitive decline emerges when age-related vulnerability is exacerbated by additional pathological processes, such as neurodegeneration, vascular injury, and inflammation [81].

Figure 12. Components of pathology of cognitive decline [82].



Neurodegeneration

Neurodegeneration plays a critical role in the transition from brain aging to cognitive decline. One of the most prominent pathological features is the accumulation of abnormal proteins, such as amyloid-β (Aβ) plaques and hyperphosphorylated tau neurofibrillary tangles, which are hallmarks of Alzheimer's disease (AD). These protein aggregates disrupt synaptic function and contribute to neuronal loss and brain atrophy. Importantly, amyloid deposition can begin 15–20 years before cognitive symptoms appear, indicating that neurodegenerative processes initiate long before clinical decline becomes noticeable. Tau pathology similarly precedes symptoms and is strongly correlated

with the severity of neurodegeneration. Synaptic loss, in particular, is a key factor in cognitive decline, as it directly affects cognitive performance, even before significant neuronal death occurs [73],[81],[82].

Vascular injury

Cerebrovascular injury is another major contributor to cognitive decline in aging. Vascular risk factors such as hypertension, diabetes, and obesity result in structural and functional changes in small cerebral vessels, leading to microangiopathy, impaired blood flow, and blood-brain barrier dysfunction. These vascular alterations contribute to chronic hypoperfusion, silent ischemic injury, and white matter damage, all of which exacerbate synaptic loss and cognitive decline. The combination of neurodegeneration and vascular injury often leads to "mixed dementia," where vascular damage accelerates the onset and progression of neurodegenerative diseases [81],[82].

Inflammaging & metainflammation

Inflammaging represents one of the key concepts in modern geroscience, referring to a state of chronic, low-grade, sterile inflammation that accompanies aging and contributes to the development of age-related chronic diseases. Unlike acute inflammation caused by infections, inflammaging is driven by a complex interaction of factors, including cellular senescence, mitochondrial dysfunction, reactive oxygen species production, gut microbiota imbalances, and increased intestinal permeability. Furthermore, chronic activation of the NLRP3 inflammasome and accumulation of dysfunctional immune cells contribute to this inflammatory state [24],[26]. This chronic inflammation exacerbates the progression of cognitive decline and other age-related diseases, such as diabetes, hypertension, and cardiovascular disease. Parallel to inflammaging, the concept of *metaflammation* was introduced to describe a chronic inflammatory response induced by metabolic disturbances, typically due to overnutrition and imbalances in energy homeostasis common in Western lifestyles. This form of inflammation is characterized by chronic activation of resident macrophages, which shift towards a pro-inflammatory M1 phenotype. The imbalance between M1 and M2 macrophage activity leads to elevated cytokine secretion (e.g., TNF-α and IL-6), exacerbating insulin resistance and promoting a chronic low-grade inflammatory state. This, in turn, accelerates the pathogenesis of metabolic disorders, cognitive decline, and frailty in older adults [24],[83].

Inflammaging and metaflammation share common mechanisms, particularly involving immune activation, altered metabolism, and unresolved inflammation. Inflammaging is driven by intrinsic factors like aging and cellular damage, while metaflammation is linked to external factors such as diet and nutrient excess. Both processes emphasize the importance of immune-metabolic pathways in age-related diseases and cognitive decline. Evolutionarily, these responses were beneficial during infection or famine but become harmful in old age or with overnutrition, leading to an "adaptive circuit malfunction.

Interaction between the three components

Neurodegeneration, cerebrovascular injury, and chronic inflammation do not act independently. Instead, they interact synergistically, amplifying their respective effects and accelerating the cognitive decline process. Inflammation can worsen neurodegeneration by impairing the brain's ability to clear toxic protein aggregates and by increasing synaptic dysfunction. Similarly, vascular injury can exacerbate neurodegenerative changes, lowering the threshold for clinical symptoms and accelerating their onset. These interactions contribute to the heterogeneity of clinical presentations in aging adults, as the combined effects of these pathologies lead to more rapid cognitive decline in some individuals [81]–[83].

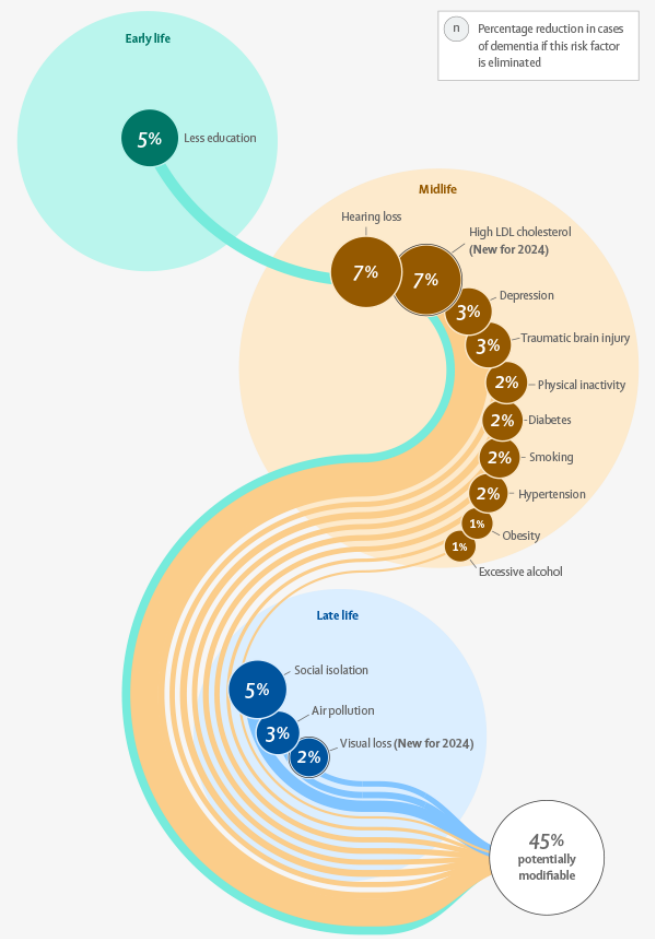
4.1.5 *From physiological aging to cognitive decline*

Brain aging is shaped not only by intrinsic biological mechanisms but also by external and potentially modifiable exposures that act across the life course. These influences operate through systemic health and vulnerability states, ultimately reducing synaptic plasticity and compensatory capacity and increasing susceptibility to pathological cognitive decline [69],[70].

A life-course perspective is crucial as dementia risk is influenced by exposures accumulated over a lifetime, with their impact varying based on timing and duration [69]. The long preclinical phase complicates interpretation, as associations in later life may reflect causality, reverse causation, or bidirectional effects. Modifiable factors, social determinants, and health states such as frailty and multimorbidity shape when symptomatic decline occurs. Exposures act over decades but are often

measured at single time points, highlighting the need for long follow-up periods to reduce reverse causation bias [69],[70],[74] (Figure 13).

Figure 13. Population attributable fraction of potentially modifiable risk factors for dementia [69].



Education is among the most consistently supported protective factors for late-life cognition. The Lancet Commission emphasizes that educational attainment and quality rather than years of schooling alone appear to drive protection, with education functioning both as a cognitive reserve factor and a social determinant shaping lifelong opportunities [69]. Higher education and cognitive stimulation throughout life, particularly from complex work, strengthen cognitive reserve and reduce dementia risk. Socioeconomic factors also contribute. However, late-life cognitive training shows limited benefits and uncertain long-term impact [69],[70].

Vascular and metabolic risk factors represent major pathways linking modifiable exposures to cognitive decline. Hypertension, diabetes, obesity, and dyslipidaemia contribute to small-vessel disease, reduced perfusion, and blood–brain barrier dysfunction, promoting chronic brain injury and neuroinflammation that can interact with neurodegenerative processes [69],[82]. Observational associations support risk reduction with vascular control, but RCT evidence is mixed. In SPRINT MIND, intensive blood pressure control reduced probable dementia non-significantly, while significantly lowering risk for MCI and combined outcomes [70],[80]. These findings suggest that effects may depend on timing, baseline vascular burden, competing risks, and the multifactorial nature of late-life cognitive decline.

Dietary patterns like the Mediterranean, DASH, and MIND diets show positive associations with cognitive health, but a MIND diet trial showed no significant effects on cognition. Weight loss in middle-aged adults with obesity improved attention and memory. While single-factor modifications haven't proven definitive in preventing dementia, these interventions are meaningful for general health and may positively impact cognitive trajectories [69],[70],[84].

Untreated sensory impairments, particularly hearing loss, are recognized as modifiable dementia risk factors. Sensory loss can increase cognitive load, promote social withdrawal, and contribute to depression, which can reduce cognitive reserve. Sensory optimization is most effective when part of broader strategies focused on social engagement and mental health [70].

Physical activity has strong observational support as a protective factor, though timing and reverse causation remain relevant considerations. Interventional evidence is most encouraging for structured programs: a multicomponent exercise intervention including strength training improved cognition, particularly in individuals with MCI, consistent with plausible vascular-metabolic, neurotrophic, and anti-inflammatory mechanisms while also preserving physical reserve [69],[80].

Smoking is consistently associated with increased dementia risk, and cessation is linked to reduced subsequent risk [70]. Alcohol evidence is mixed, with uncertainty around light-to-moderate intake and more consistent concern for heavy use or alcohol use disorders [69]. Sleep disturbances are also associated with increased risk of all-cause dementia, AD, and vascular dementia; treatment of obstructive sleep apnea may reduce incidence in observational data, although trial evidence is still

lacking regarding biomarker reversal. Sleep therefore remains both a plausible intervention target and a domain strongly intertwined with comorbidity, mood, and early neurodegenerative changes [69].

Psychiatric and social factors, such as depression and social isolation, contribute to dementia risk. Depression may predict future dementia, act as a prodromal symptom, or occur concurrently, but no RCT has proven that treating depression prevents dementia. Depression worsens cognitive complaints, reduces protective behaviours, and contributes to functional decline. Social isolation increases dementia risk, though causality is unclear [69].

In older adults, dementia risk is influenced by the cumulative burden of chronic diseases and functional vulnerability. The Lancet Commission highlights that multimorbidity, especially when chronic illnesses begin in midlife, increases dementia risk. Frailty, common in older adults and more prevalent in women, is linked to poorer neuropsychological performance and higher dementia risk. Multimorbidity, particularly cardiovascular and cardiometabolic diseases, has a significant impact on dementia risk. Additionally, frailty independently contributes to dementia risk, intensifying the effects of neuropathology. These findings underscore the importance of incorporating physical function and systemic health markers in geriatric care to assess vulnerability and resilience to cognitive decline.

4.1.6 *Diagnostic pathway of cognitive decline*

A timely and accurate diagnosis of cognitive impairment and dementia is a cornerstone of effective care. Diagnosis enables identification of potentially reversible contributors, clarification of the underlying etiologic substrate, prognostic counselling, and the initiation of tailored management strategies, including support for patients and caregivers and planning for future care needs [69]. The first step in diagnosing dementia is not a test but recognizing the possibility that dementia may be developing. However, this "trigger phase" is often delayed due to social and structural factors. Family members often help by taking on tasks like managing finances or household chores, which can hide functional decline and delay recognition. Barriers to diagnosis include stigma, lack of knowledge, fear, and difficulties accessing proper services. On the other hand, recognizing symptoms, having prior knowledge of healthcare options, and strong support networks can help facilitate the diagnosis. Once suspicion is raised, evaluation begins with a structured clinical assessment. The aim is to determine

whether cognitive impairment is present, characterize its pattern and severity, identify functional consequences, and generate a differential diagnosis that includes neurodegenerative, vascular, psychiatric, metabolic, sensory, and medication-related contributors. Because cognitive decline frequently reflects multiple coexisting pathologies in older adults, diagnostic evaluation must remain syndromic and integrative. Key elements include: (i) detailed history of cognitive symptoms (onset, progression, and domain-specific complaints), (ii) functional assessment (ADL/IADL changes), (iii) informant history when available, (iv) review of medical comorbidities and medications, (v) assessment of mood, sleep, and sensory function, and (vi) neurological and general physical examination. This step is essential to avoid prematurely attributing impairment to AD when alternative or contributory causes are present, particularly depression, sleep disorders, sensory impairment, and delirium, each of which can mimic or exacerbate cognitive symptoms [70].

Validated screening instruments support early detection and triage. Common tools include Mini-Cog, the Mini-Mental State Examination (MMSE), and the Montreal Cognitive Assessment (MoCA) [70]. These instruments differ in sensitivity and domain coverage. MMSE provides a broad assessment of orientation, attention, calculation, recall, and language but is often less sensitive to early impairment. Screening is not diagnostic; rather, it informs the need for deeper domain-specific testing and helps stage severity.

Neuropsychological testing plays a critical role in the comprehensive assessment of cognitive decline. While there is no one-size-fits-all battery of tests, the selection of neuropsychological assessments should be tailored to evaluate the broad range of cognitive domains that could be affected, such as memory, attention, executive functions, language, and visuospatial abilities. These assessments are designed to capture subtle changes in cognitive functioning that may not be evident through routine clinical observation. Importantly, these tests must consider individual factors such as age, educational background, and sometimes sex, as these variables can significantly influence baseline cognitive performance. Additionally, neuropsychological tests have specific cutoff points that are used to identify cognitive impairment relative to expected norms for a person's demographic group. These cutoff values help ensure that the results reflect true cognitive deficits rather than variations that can occur due to normal age-related changes or individual differences [70],[85].

Given the syndromic nature of cognitive impairment and dementia, as well as the common coexistence of both mental and physical health conditions especially in older adults, assessing physical capabilities and body composition can offer crucial insights. This approach not only complements cognitive evaluations but also helps capture the broader picture of health, providing a more comprehensive understanding of an individual's overall functioning and potential vulnerability to further decline [86]. Neuroimaging plays a key role in diagnosing cognitive decline by excluding other causes, assessing vascular damage, supporting the diagnosis, and monitoring treatment effects. CT scans are widely accessible and can identify structural issues like vascular changes and atrophy. MRI offers more precision, especially for detecting hippocampal and temporal lobe atrophy linked to AD. It is primarily used to rule out other causes rather than confirm AD, though atrophy patterns can support clinical suspicion. Functional imaging, like FDG-PET, helps assess neurodegeneration by detecting brain hypometabolism, but its specificity for preclinical AD is limited. More specific imaging, such as amyloid-PET and tau-PET, enables in-vivo assessment of AD pathology. Modern diagnostic frameworks integrate biomarkers, including CSF and blood-based assays, within the ATN model (amyloid, tau, neurodegeneration), offering biological staging independent of symptoms. However, biomarker positivity does not always indicate dementia, especially in older adults, and should be interpreted in clinical context with a focus on concordant biomarkers [73].

4.1.7 *Outcomes of cognitive decline*

Outcomes in cognitive impairment and dementia are multidimensional and cannot be captured by cognitive test scores alone; clinically meaningful trajectories include functional change, neuropsychiatric symptoms, caregiver burden, and major health events such as hospitalization, institutionalization, delirium and mortality [70]. The progression of AD follows a continuum, starting with biomarker positivity, followed by subjective complaints, mild cognitive impairment MCI, and ultimately dementia. However, this progression is not inevitable, and the rate of transition and cognitive decline varies depending on factors such as age, genetic predisposition, comorbidities, and cognitive reserve [69],[74].

In cases of MCI, cognitive decline is often subtle, and functional impairment may not be significant enough to disrupt daily life. Longitudinal monitoring to track cognitive changes, though these measures capture only part of the overall clinical burden, with varying sensitivity across stages and settings [69],[70]. The primary outcomes in MCI are related to functional decline, particularly in instrumental activities of daily living (IADLs), and whether these individuals transition into dementia. In contrast, dementia progression is marked by more significant impairment in both cognitive and functional domains, with increased supervision needed for activities of daily living (ADLs). For these individuals, symptomatic treatments may provide modest cognitive benefits and delay institutionalization, while also reducing caregiver burden [70]. Neuropsychiatric symptoms are central to the dementia trajectory: mild behavioural impairment (MBI) may appear in the pre-dementia phase, while behavioural and psychological symptoms of dementia (BPSD), such as apathy, agitation, delusions, and sleep disruptions, often persist, driving distress, urgent care needs, and care transitions Therefore, prognosis in geriatric outpatient cohorts should involve an integrated assessment that considers cognitive phenotype, functional status, neuropsychiatric symptoms and systemic vulnerability factors such as inflammation and physical reserve [77],[78].

4.1.8 *Treatment of cognitive decline*

Non-pharmacological care is the backbone of dementia management across the full spectrum from MCI to established dementia because it directly targets outcomes that matter most in practice: function, safety, neuropsychiatric symptoms, caregiver burden, and quality of life [70],[73]. Its role is amplified in geriatric outpatient populations, where frailty, multimorbidity, sensory impairment, and polypharmacy are common and medication-related harms are a major concern; moreover, many patients are not eligible for disease-modifying therapies and pharmacologic benefits may be modest at the individual level. Effective implementation requires a person-centred approach that integrates clinical assessment, patient goals, caregiver context, and environmental factors. Diagnosis enables anticipatory planning and safety assessment while addressing modifiable contributors such as hearing loss, sleep disturbance, depression, medication side effects, and delirium risk [69]. Core interventions for cognitive decline include personalized cognitive and functional strategies such as compensatory tools, routines,

and environmental adjustments. Physical activity and rehabilitation programs, often incorporating strength training, aim to preserve mobility, reduce falls, and combat frailty. Sensory optimization is also key to improving communication and engagement. Additionally, sleep management, non-pharmacological circadian interventions, and nutrition-focused strategies help prevent malnutrition, sarcopenia, and metabolic dysregulation, while preserving lean mass [70],[80]. Neuropsychiatric symptoms are first addressed through structured assessments and non-pharmacological methods like environmental modifications and activity adjustments, with caregiver-focused programs effectively reducing both symptom severity and caregiver burden. Finally, delirium prevention and the minimization of high-risk medications are critical to preventing cognitive decline and improving outcomes [70].

Pharmacological treatment has two main aims: symptomatic therapy to modestly improve or stabilize cognition and function, and disease-modifying therapy, currently most developed for AD, intended to slow progression in selected early-stage patients[74]. Decisions must be individualized by etiology, stage, comorbidity burden, and patient–caregiver priorities, particularly in geriatric cohorts where multimorbidity and polypharmacy strongly shape risk–benefit profiles.

Symptomatic AD treatments include cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and memantine, which do not reverse underlying neurodegeneration but can yield modest benefits; response is heterogeneous, and some evidence links these agents to delayed institutionalization and reduced caregiver burden [70],[73]. The major recent shift has been the introduction of anti-amyloid monoclonal antibodies (e.g., lecanemab and donanemab, with prior controversy around aducanumab), which reduce amyloid and can slow decline in selected patients with MCI due to AD or mild AD dementia, although average benefits are modest and uncertain at the individual level [80]. Their real-world use is constrained by substantial eligibility and monitoring requirements, amyloid confirmation, baseline MRI assessment, APOE ε4–related risk considerations, repeated infusions, and serial MRI surveillance because of the risk of amyloid-related imaging abnormalities (ARIA), often asymptomatic but potentially serious.

For behavioural and psychological symptoms of dementia, pharmacotherapy is reserved for severe or dangerous symptoms refractory to non-pharmacological approaches and should be time-limited and

goal-directed, ideally preceded by deprescribing of ineffective or harmful psychotropics. Antipsychotics have modest efficacy but important safety concerns, including falls and mortality risk, and potential cognitive worsening, necessitating cautious use [77],[78]. Finally, pharmacological options for non-AD dementias remain limited, with few approvals and generally weak evidence for off-label use; this reinforces the need for etiologically informed prescribing within a broader integrated care strategy.

4.1.9 *Exploring the cognitive continuum: identification and multidimensional profiling*

Cognitive decline exists on a continuum, ranging from healthy aging to subjective memory complaints (SCC), mild cognitive impairment, and eventually to dementia. This continuum highlights the importance of early detection and accurate identification of individuals at various stages of cognitive decline. In addition to neurodegenerative conditions, certain prevalent conditions, such as depression, can present with symptoms mimicking dementia, leading to what is known as "pseudodementia." This distinction is crucial, as depression-related cognitive impairment may be reversible with appropriate intervention, unlike neurodegenerative diseases.

Assessing individuals along the cognitive continuum requires a comprehensive, multidimensional approach, as cognitive decline is influenced by various factors. This involves combining cognitive evaluations with assessments of physical function, mental health, and other biological markers. Due to the complexity and overlap of these conditions, an integrated strategy is crucial for early identification of at-risk individuals and to guide appropriate interventions.

Currently, therapeutic options for cognitive decline remain limited highlighting the importance of early identification. Given the complexity of these conditions and their overlapping features, it is crucial to maximize the effectiveness of early detection. Integrating multidimensional profiling allows for more accurate assessment across the cognitive continuum, from normal aging to advanced dementia. This approach ensures proper identification and stratification, leading to more targeted interventions and improved care outcomes.

4.2 Aims

To characterise the neurocognitive spectrum in a geriatric outpatient cohort through a multidimensional approach integrating neuropsychological, inflammatory, metabolic, body composition, and bioenergetic domains, and to evaluate the diagnostic accuracy of each domain, individually and in combination, for the discrimination of clinically relevant pairwise neurocognitive comparisons.

1. Domain 1 - Neuropsychological profiling: To characterise neuropsychological performance across cognitive domains and mood by diagnostic group, and to evaluate the discriminative ability of individual test scores across cognitive profiles.

2. Domain 2 - Body composition and muscle strength: To assess body composition parameters and handgrip strength across diagnostic groups, to classify sarcopenia prevalence, and to evaluate the diagnostic contribution of body composition indices across the five pairwise comparisons.

3. Domain 3 - Metabolic and haematological profile: To evaluate biochemical and haematological markers across diagnostic groups and assess their individual discriminative performance across the pairwise comparisons.

4. Domain 4 - Inflammatory cytokine profiling: To characterise the plasma cytokine and chemokine profile across the neurocognitive spectrum, to derive composite inflammatory indices, and to evaluate their discriminative performance across the cognitive continuum.

5. Integrated multidimensional predictive modelling: To build progressive logistic regression models for each pairwise comparison by sequentially integrating variables from each domain; to quantify the incremental discriminative contribution of each domain; and to formally test whether the full integrated model outperforms the best single-domain predictor.

6. Bio-cognitive profiling and patient subgrouping: To construct a multidimensional representation of patient heterogeneity integrating all four domains, to validate the biological and cognitive axes of variability, and to identify natural patient subgroups through unsupervised clustering.

7. PBMC bioenergetics (exploratory): To explore mitochondrial respiration and glycolytic capacity of peripheral blood mononuclear cells across all diagnostic groups, including Healthy controls as a biological reference.

4.3 Methods

4.3.1 *Experimental design*

This study conducted a retrospective analysis of data from 97 patients who were referred to the Memory and Mood Disorders Outpatient Clinic at Policlinico Riuniti, Foggia between November 2023 and January 2026.

4.3.2 *Study population*

Patients aged 65 and older were selected from day service activities. Inclusion in the study was voluntary, with prior informed consent obtained from all participants. Participation did not affect the established diagnostic and therapeutic pathways for the individuals involved, and they were informed of their right to withdraw consent at any time without providing justification to healthcare staff, ensuring their autonomy in decision-making. Only individuals meeting the eligibility criteria namely, aged 65 or older and having signed the informed consent, were included. Exclusion criteria included patients who had undergone cognitive stimulation interventions in the six months prior to the study, those who had been hospitalized or experienced significant trauma in the past three months, and individuals who did not sign the informed consent.

4.3.3 *Clinical assessment*

All patients underwent a comprehensive evaluation, starting with a medical visit during which demographic and anthropometric data were collected. Additionally, information on lifestyle habits, comorbidities, and current medications was gathered. Specifically, sociodemographic details such as age, gender, marital status, education and living place were recorded. Lifestyle factors, including smoking, alcohol consumption, and physical activity levels, were also assessed. Other key data collected included the reasons for the visit and comorbidities such as atrial fibrillation, myocardial infarction, pacemaker use, valvulopathies, chronic heart failure, hypertension, dyslipidemia, carotid atherosclerosis, diabetes, liver disease, thyroid dysfunction, osteoporosis, osteopenia,

leukoencephalopathy, as well as any history of active or past neoplasms, and visual or auditory impairments (including the use of glasses or hearing aids).

4.3.4 *Domain 1 - Cognitive, mood and functional assessment*

The neuropsychological evaluation was conducted in an outpatient setting under standardized conditions. To ensure accurate results, the testing environment was optimized with proper lighting, a quiet atmosphere, and without the need for fasting. Prior to administering the tests, the patient's ability to see, read, and comprehend was assessed to ensure that sensory and cognitive limitations would not interfere with the evaluation. Additionally, breaks were provided as needed to maintain focus and avoid fatigue during the assessment. This approach was aimed at ensuring that the results reflected the patient's cognitive abilities rather than external factors such as discomfort or distractions.

The neuropsychological evaluation consisted of the administration of thirteen tests, designed to assess a range of cognitive domains, depressive symptoms and autonomy (Table 6).

Table 6. Test performed for cognitive, mood and functional assessment.

Test	Main cognitive domains explored
Mini Mental State Examination (MMSE) [87]	Global cognitive function
Babcock Story Recall Test [88]	Long-term memory
Construction Apraxia [89]	Praxis
Verbal Fluency (Memory) [90]	Semantic memory
Clock Drawing Test (CDT) [91]	Praxis and visuospatial memory
Maze Test [92]	Visual search and executive skills
Frontal Assessment Battery (FAB) [93]	Executive functions
Trail Making Test A (TMT-A) and B (TMT-B) [94]	Visual search and task-switching
Attentive Matrix Test [95]	Visual attention
Rey's 15-Word Test [96]	Short and long-term verbal memory
ADL (Activities of Daily Living) [97]	Daily living autonomy
IADL (Instrumental Activities of Daily Living) [98]	Instrumental autonomy
Geriatric Depression Scale short form (GDS-sf) [99]	Depression symptoms in the elderly

Each test was administered with specific cut-off scores to identify deficits across cognitive domains, following standardized criteria. The short form of the Geriatric Depression Scale (GDS-sf), composed of 15 questions, was used to screen for depressive symptoms. Functional abilities were assessed using the ADL and IADL scales, consisting of 6 and 8 items, respectively.

4.3.5 *Domain 2 - Body composition and muscle strength*

Body composition was assessed using bioelectrical impedance analysis (BIA), a non-invasive method that estimates the proportions of fat mass, lean body mass, and total body water by measuring the resistance of body tissues to a low-level alternating electrical current. BIA is particularly informative in aging populations, where changes in body composition including progressive loss of lean mass and relative increase in fat mass, are closely associated with frailty, functional decline, and adverse health outcomes. Measurements were performed under standardised conditions: patients were assessed in the supine position with arms and legs uncrossed; they were instructed to fast for at least 4 hours prior to the assessment, to abstain from vigorous physical activity for at least 12 hours, and to avoid alcohol and diuretics for 24 hours before the test. Electrodes were placed on the dorsal surface of the hands and feet, and impedance measurements were recorded and adjusted for age, sex, height, and weight using dedicated analysis software. The following parameters were derived: fat mass (kg and %), fat-free mass (kg), lean soft tissue (kg), skeletal muscle mass (kg), body cell mass (kg), phase angle (°), fat mass index (%), and fat-free mass index (%).

Muscle strength was assessed using a calibrated handgrip dynamometer. Patients were seated with the arm at the side and the elbow flexed at 90 degrees. The dynamometer was placed alternately in the right and left hand, and patients were instructed to squeeze the handle as hard as possible for approximately 3–5 seconds with a steady, consistent force. Three measurements were obtained for each hand in alternating sequence, with rest intervals between repetitions to minimise muscle fatigue. Maximum handgrip strength was defined as the highest value across all six measurements. To account for biological differences between sexes and for the contribution of body muscle mass to absolute grip force, a sex- and muscle mass-adjusted handgrip residual was derived as the unstandardised residual from a linear regression of maximum handgrip strength on sex and skeletal muscle mass.

The Appendicular Skeletal Muscle Index (ASMI) was computed as skeletal muscle mass (kg) divided by height in metres squared (kg/m²). Sarcopenia was classified according to the revised European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria [100]. Low handgrip strength was defined as less than 27 kg in men and less than 16 kg in women; low ASMI as less than 7.0 kg/m² in men and less than 5.5 kg/m² in women. Sarcopenia was diagnosed as the co-occurrence of both low handgrip strength and low ASMI.

4.3.6 *Domain 3 - Metabolic and Haematological Profile*

A fasting venous blood sample was collected at the beginning of the study visit, prior to any clinical or cognitive assessment. All laboratory analyses were performed by the standardised hospital clinical laboratory, Policlinico Riuniti di Foggia. Samples were processed and analysed on the same day of collection.

The following parameters were assessed: fasting plasma glucose, glycated haemoglobin (HbA1c), total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, estimated glomerular filtration rate (eGFR), serum creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), 25-hydroxyvitamin D, vitamin B12, serum folate, thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), white blood cell count (WBC), neutrophils, lymphocytes, monocytes, and platelets. Two composite haematological ratios were derived: the Neutrophil-to-Lymphocyte Ratio (NLR) and the Platelet-to-Lymphocyte Ratio (PLR), computed as neutrophil-to-lymphocyte and platelet-to-lymphocyte counts respectively, restricted to observations with lymphocyte count greater than zero.

4.3.7 *Domain 4 - Cytokines, chemokines, and growth factors*

Serum samples were collected from all participants, and standard hematochemical tests were performed to assess various biomarkers and metabolic parameters. Moreover, serum concentrations of cytokines, chemokines, and growth factors were measured using the Luminex® 200™ system. Specifically, the Human Cytokine/Chemokine/Growth Factor Panel was used to assess 46 analytes (Table 7; Figure 14). Serum samples, thawed and processed according to the manufacturer's

instructions, were incubated with microspheres conjugated with specific antibodies, followed by the addition of biotinylated secondary antibody and streptavidin-PE. Data analysis was performed using the xPONENT software, comparing the readings to a standard curve generated from known concentration samples, allowing for interpolation of actual concentrations in biological samples. These biomarkers are involved in immune responses, inflammation, tissue repair, and cell proliferation, contributing to various physiological and pathological processes within the body. Imbalances or disruptions in these factors may influence the development of diseases such as autoimmune conditions, cancer, cardiovascular diseases, and neurodegenerative disorders.

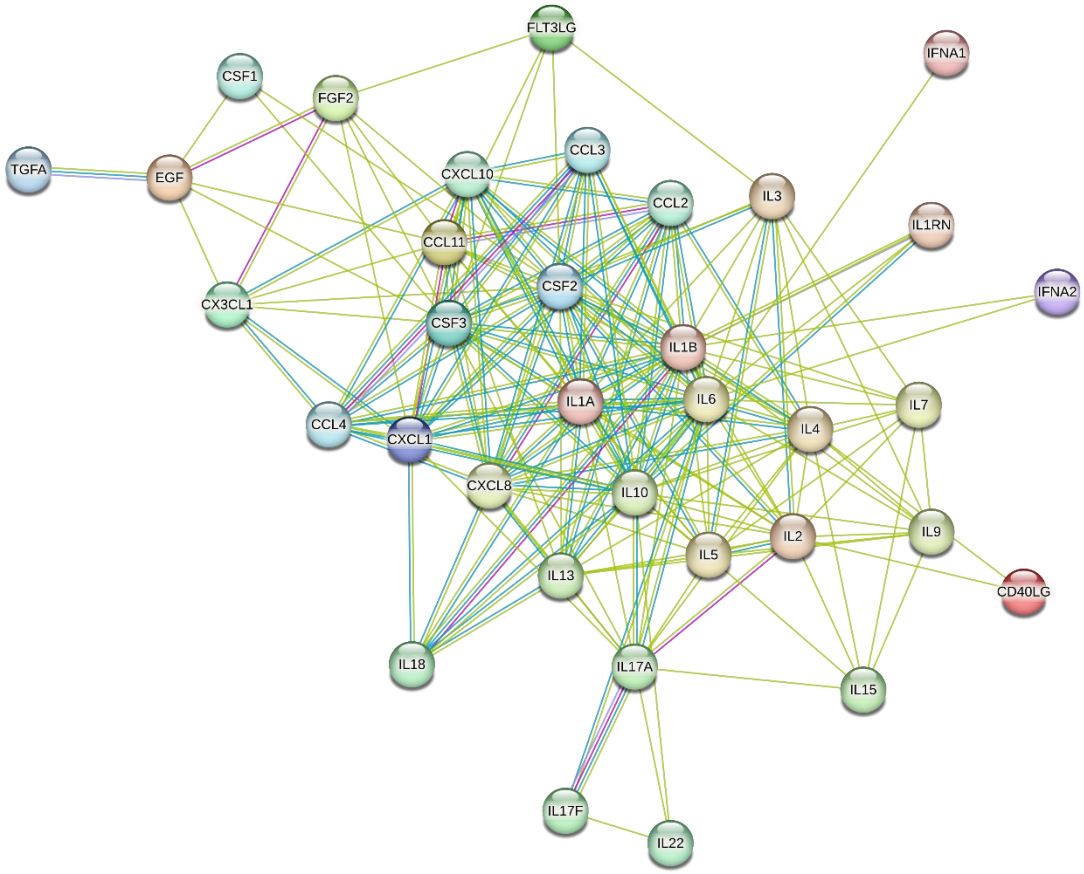
Table 7. Human Cytokines, Chemokines, and Growth Factors Analyzed and Their Functions. Modified from string-db.org/.

scD40L	Costimulates T-cell proliferation and cytokine production. Induces the activation of NF-kappa-B. Induces the activation of kinases MAPK8 and PAK2 in T-cells. Mediates B-cell proliferation in the absence of co-stimulus as well as IgE production in the presence of IL4.
EGF	Stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro and of some fibroblasts in cell culture.
Eotaxin	In response to the presence of allergens, this protein directly promotes the accumulation of eosinophils, a prominent feature of allergic inflammatory reactions.
FGF-2	Plays an important role in the regulation of cell survival, cell division, cell differentiation and cell migration. Can induce angiogenesis.
FLT3L	Stimulates the proliferation of early hematopoietic cells by activating FLT3. Synergizes well with several other colony stimulating factors and interleukins.
Fractalkine	The soluble form is chemotactic for T-cells and monocytes and not for neutrophils.
G-CSF	Controls the production, differentiation, and function of 2 related white cell populations of the blood, the granulocytes and the monocytes-macrophages. This CSF induces granulocytes; Belongs to the IL-6 superfamily.
GM-CSF	Stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes.
CXCL1	Has chemotactic activity for neutrophils. May play a role in inflammation and exerts its effects on endothelial cells in an autocrine fashion.
IFNA	Produced by macrophages, IFN-alpha have antiviral activities.
IFN	Produced by macrophages, has antiviral activities. Belongs to the alpha/beta interferon family.
Hematopoietin-1	Produced by activated macrophages, stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. Involved in the inflammatory response, being identified as endogenous pyrogens, stimulates the release of prostaglandin.
IL1F2	Potent proinflammatory cytokine. Induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B- cell activation and antibody production, and fibroblast proliferation and collagen production. Promotes Th17 differentiation of T-cells.
IL-1RA	Inhibits the activity of interleukin-1 by binding to receptor IL1R1 and preventing its association with the coreceptor IL1RAP for signalling.
IL-2	Produced by T-cells in response to antigenic or mitogenic stimulation, required for T-cell proliferation and other activities crucial to regulation of the immune response.

	Can stimulate B-cells, monocytes, lymphokine-activated killer cells, natural killer cells, and glioma cells.
IL-3	Acts in hematopoiesis by controlling the production, differentiation, and function of the granulocytes and the monocytes-macrophages.
IL-4	Participates in at least several B-cell activation processes as well as of other cell types. It is a costimulator of DNA-synthesis, induces the expression of class II MHC molecules on resting B-cells, enhances both secretion and cell surface expression of IgE and IgG1.
IL-5	Induces terminal differentiation of late- developing B-cells to immunoglobulin secreting cells.
IL-6	Inducer of the acute phase response, plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS.
IL-7	Hematopoietic growth factor capable of stimulating the proliferation of lymphoid progenitors. It is important for proliferation during certain stages of B-cell maturation.
IL-8	Chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes. It is also involved in neutrophil activation. It is released from several cell types in response to an inflammatory stimulus.
IL-9	Supports IL-2 independent and IL-4 independent growth of helper T-cells; Belongs to the IL-7/IL-9 family.
IL-10	Major immune regulatory cytokine that acts on many cells of the immune system where it has profound anti-inflammatory functions, limiting excessive tissue disruption caused by inflammation.
NKSF	Aka Interleukin-12 subunit beta; acts as a growth factor for activated T and NK cells, enhances the lytic activity of NK/lymphokine-activated killer cells, and stimulates the production of IFN-gamma by resting PBMC. Belongs to the IL-12B family.
IL-13	Inhibits inflammatory cytokine production. Synergizes with IL2 in regulating interferon-gamma synthesis. May be critical in regulating inflammatory and immune responses.
IL-15	Stimulates the proliferation of T-lymphocytes. Stimulation by IL15 requires interaction of IL15 with components of the IL2 receptor, including IL2RB and probably IL2RG but not IL2RA.
IL-17A	Ligand for IL17RA and IL17RC. Involved in inducing stromal cells to produce proinflammatory and hematopoietic cytokines; belongs to the IL-17 family.
IL-27	Bone marrow-derived monocyte and paracrine-acting protein that promotes cardiac myocyte survival and adaptive angiogenesis for cardiac protection and/or repair after myocardial infarction (MI).
IL-17F	Involved in stimulating the production of other cytokines such as IL6, IL8 and CSF2, and in regulation of cartilage matrix turnover. Also involved in stimulating the proliferation of peripheral blood mononuclear cells and T-cells and in inhibition of angiogenesis. Plays a role in the induction of neutrophilia in the lungs and in the exacerbation of antigen-induced pulmonary allergic inflammation.
IL-18	A proinflammatory cytokine primarily involved in polarized T- helper 1 (Th1) cell and natural killer (NK) cell immune responses (Probable). Synergizes with IL12/interleukin-12 to induce IFNG synthesis from T-helper 1 (Th1) cells and natural killer (NK) cells (Probable). Belongs to the IL-1 family.
IL-22	Contributes to the inflammatory response in vivo.
IP-10	Involved in chemotaxis, differentiation, and activation of peripheral immune cells, regulation of cell growth, apoptosis and modulation of angiostatic effects. Plays an important role during viral infections by stimulating the activation and migration of immune cells to the infected sites.
MCP-1	Acts as a ligand for C-C chemokine receptor CCR2. Exhibits a chemotactic activity for monocytes and basophils but not neutrophils or eosinophils. May be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis.

MCP-3	Chemotactic factor that attracts monocytes and eosinophils, but not neutrophils. Augments monocyte anti-tumor activity.
M-CSF	Plays an essential role in the regulation of survival, proliferation and differentiation of hematopoietic precursor cells, especially mononuclear phagocytes, such as macrophages and monocytes. Promotes the release of proinflammatory chemokines, plays an important role in innate immunity and in inflammatory processes, regulation of osteoclast proliferation and differentiation, bone resorption, and is required for normal bone development.
MDC	Probable ligand for integrin in the brain.
CXCL9	Cytokine that affects the growth, movement, or activation state of cells that participate in immune and inflammatory response. Chemotactic for activated T-cells. Binds to CXCR3; Belongs to the intercrine alpha (chemokine CxC) family.
CCL3	Monokine with inflammatory and chemokinetic properties. Binds to CCR1, CCR4 and CCR5.
CCL4	Monokine with inflammatory and chemokinetic properties. Binds to CCR5. The processed form MIP-1-beta(3-69) retains the abilities to induce down-modulation of surface expression of the chemokine receptor CCR5 and to inhibit the CCR5-mediated entry of HIV-1 in T-cells.
Platelet-derived Growth Factor	Growth factor that plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. Potent mitogen for cells of mesenchymal origin. Required for normal proliferation and recruitment of pericytes and vascular smooth muscle cells in the central nervous system, skin, lung, heart and placenta, for normal blood vessel development, and for normal development of kidney glomeruli. Plays an important role in wound healing.
TGFA	It is a mitogenic polypeptide that can bind to the EGF receptor/EGFR and to act synergistically with TGF beta to promote anchorage-independent cell proliferation in soft agar.
Cachectin	Mainly secreted by macrophages, can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, under certain conditions it can stimulate cell proliferation and induce cell differentiation. Impairs regulatory T-cells (Treg) function in individuals with rheumatoid arthritis via FOXP3 dephosphorylation.
TNFB	Cytokine that in its homotrimeric form binds to TNFRSF1A/TNFR1, TNFRSF1B/TNFBR and TNFRSF14/HVEM. In its heterotrimeric form with LTB binds to TNFRSF3/LTBR. Lymphotoxin is produced by lymphocytes and is cytotoxic for a wide range of tumor cells in vitro and in vivo.
VEGF-A	Growth factor active in angiogenesis, and endothelial cell growth, stimulating their proliferation and migration and has effects on the permeability of blood vessels. May function in angiogenesis of the venous and lymphatic vascular systems during embryogenesis, and in the maintenance of differentiated lymphatic endothelium in adults.

Figure 14. Network of Human Cytokines, Chemokines, and Growth Factors Analyzed. Source: string-db.org/.



Colored nodes: query proteins and first shell of interactors. Lines, colours: fuchsia, experimentally determined; light blue: from curated databases; light green: textmining.

4.3.8 *PBMC isolation*

Peripheral blood was collected in VACUTAINER tubes (BD, Franklin Lakes, NJ) containing lithium heparin as an anticoagulant and processed within 30 minutes. Aliquots of 20 mL of whole blood were diluted 1:1 with EasySep Buffer (Stemcell Technologies), layered onto 15 mL of Lymphoprep (Stemcell Technologies), and centrifuged at 1200 g for 10 minutes. The mononuclear cell layer was collected and resuspended in EasySep Buffer. Cells were subjected to two sequential washes by centrifugation: 250 g for 10 minutes and then 200 g for 10 minutes. The pellet was immediately processed for bioenergetic analysis.

PBMC metabolic activity was assessed by measuring two parameters: proton efflux rate (PER), indicating glycolysis, and oxygen consumption rate (OCR), reflecting mitochondrial respiration. The Seahorse XF HS Mini Analyzer (Agilent Technologies) was used for measurements.

Each well of a pre-treated microplate (Agilent Technologies) was seeded with 1×10^5 cells in duplicate. Glycolytic activity was monitored by measuring the PER using the Seahorse XF Glycolytic Rate Assay Kit, while mitochondrial respiratory capacity (OCR) was measured with the Seahorse XF Cell Mito Stress Test Kit. Data were analyzed with Agilent Seahorse Analytics software, and the results were normalized to total cell protein content measured via BCA assay (Thermo Fisher Scientific).

4.3.9 *Cognitive diagnosis and diagnostic categories*

Diagnostic classification was performed by an experienced clinical team, based on a comprehensive multimodal evaluation conducted during a dedicated cognitive day-service assessment. The diagnostic process integrated the following sources of information: presenting symptoms and reason for referral; detailed medical history including current and past neurological, psychiatric, cardiovascular, and metabolic conditions; complete pharmacological history with particular attention to medications potentially affecting cognitive function; available instrumental investigations (neuroimaging, electroencephalography, and other diagnostic workup performed prior to or during the day-service); standardised neuropsychological assessment (see Domain 1); and longitudinal clinical information from previous outpatient visits where available.

Diagnostic categories were assigned according to internationally validated criteria. Healthy controls were defined as individuals with no subjective cognitive complaints, normal neuropsychological performance, and no active neurological or psychiatric condition. Subjective Cognitive Complaint (SCC) was defined as the presence of self-reported cognitive concerns in the absence of objective neuropsychological impairment, consistent with current SCC framework criteria [101]. Single-domain and multiple-domain Mild Cognitive Impairment (sMCI and mMCI) were diagnosed according to the National Institute on Aging–Alzheimer's Association (NIA-AA) guidelines [102], requiring objective cognitive impairment in one or more domains with preserved functional independence. Major

Neurocognitive Disorder (NCD) was diagnosed according to DSM-5 criteria [103], requiring evidence of significant cognitive decline from a previous level of performance in one or more cognitive domains, with interference in everyday activities. Depressive Syndrome was diagnosed according to DSM-5 criteria for major depressive disorder or persistent depressive disorder, with particular attention to the differential diagnosis with early neurocognitive conditions given the known overlap between late-life depression and prodromal dementia [103].

Five pairwise comparisons were pre-specified to reflect clinically relevant transitions along the neurocognitive continuum. SCC vs sMCI and SCC vs mMCI were selected to investigate the boundary between subjective complaint without objective impairment and early objective cognitive decline, representing the earliest and most diagnostically challenging distinction in memory clinic practice. mMCI vs NCD was selected to capture the transition from mild to major neurocognitive disorder, a clinically critical threshold with direct implications for treatment planning and prognosis. Depressive Syndrome vs mMCI and Depressive Syndrome vs NCD were included to address the well-recognised diagnostic overlap between late-life depression and neurocognitive conditions, given that cognitive symptoms are frequently present in depressive disorders and may precede, mimic, or co-occur with neurodegenerative disease.

4.3.10 *Statistical Analysis*

The Healthy group was excluded from all primary comparative analyses, with the exception of baseline characteristics evaluation and bioenergetics, where it was retained as a biological reference. First, given that all continuous variables violated the assumption of normality (Shapiro-Wilk test), a fully non-parametric analytical approach was adopted throughout. Descriptive statistics are presented as mean ± standard deviation (SD) for continuous variables and as counts and percentages for categorical variables. Group differences in continuous variables were assessed using the Kruskal-Wallis test, with Bonferroni correction applied according to the number of variables tested per domain. Post-hoc pairwise comparisons were performed using the Wilcoxon rank-sum test with Bonferroni correction for 10 comparisons, requiring a minimum cell size of $n \geq 3$. Categorical variables were compared using Pearson chi-square or Fisher's exact test. Associations between domain-specific

variables and neuropsychological performance were evaluated using Spearman rank correlation. Discriminative performance was assessed using ROC analysis (DeLong's method) for the five pre-specified pairwise comparisons. When AUC was below 0.5, the complement (1−AUC) was used with the predictor direction inverted. For the cytokine panel (Domain 4), all 45 cytokines were log(x+1) transformed prior to analysis. Principal Component Analysis (PCA) with varimax rotation was applied to the 14 cytokines reaching global significance, yielding four interpretable components. Factor scores were extracted after rotation and used in subsequent Kruskal-Wallis, correlation, and ROC analyses.

To quantify the additive discriminative value of integrating information across domains, a series of progressive Firth-penalized logistic regression models (M1 through M5) was fitted for each pairwise comparison. Given small sample sizes per comparison (n=30–46), Firth's method was chosen to reduce bias under conditions of quasi-complete separation. Models were built sequentially: M1 (demographic baseline: age, sex, education), M2 (+best neuropsychological test), M3 (+handgrip residual), M4 (+best metabolic predictor, where applicable), and M5 (+best cytokine principal component). Incremental discriminative gain (ΔAUC) was computed between consecutive models, and Events Per Variable (EPV) was monitored throughout. Internal validity of the final M5 models was assessed through bootstrap resampling (B=200 for early-stage comparisons; B=50 for advanced-stage comparisons, where near-complete separation in resampled datasets precluded a higher number of replications), yielding bootstrap-corrected AUC estimates virtually identical to the apparent values across all five comparisons (optimism ≈ 0.000), with all replications successfully converging. The DeLong test was used to formally compare the AUC of the best single neuropsychological test against the full M5 integrated model for each comparison. To characterise patient subgroups and provide a spatially interpretable representation of multidomain heterogeneity, two complementary unsupervised analyses were performed on the non-Healthy subsample. Hierarchical cluster analysis (Ward's linkage, squared Euclidean distance) was applied to z-standardized variables from the biological profile alone (Cluster V1: cytokine PC scores and handgrip residual) and from the full multidomain profile (Cluster V2: ten variables spanning all four domains). The optimal number of clusters was evaluated using the Calinski-Harabasz pseudo-F statistic. Multidimensional scaling (MDS) was applied to the same ten variables used in Cluster V2, retaining two dimensions. Dimension interpretation was validated

through Spearman correlations between MDS axes and all input and neuropsychological variables. An exploratory logistic overlay was used to assess the ability of the two MDS dimensions to predict NCD, interpreted as hypothesis-generating given borderline EPV. Mitochondrial respiration and glycolytic function of peripheral blood mononuclear cells were assessed using Seahorse XF technology. Unlike all other domains, the Healthy group was deliberately included as a biological reference for cellular metabolic profiling. Results are presented as mean ± interquartile range (IQR, p25–p75), no statistical analysis was performed given the small subsample. All analyses were performed in Stata 15 (StataCorp, College Station, TX, USA).

4.4 Results

Baseline characteristics of the study population are reported in Table 8. A total of 97 geriatric outpatients (mean age 76.6 ± 7.0 years; 55% female) were included. The sample comprised six diagnostic groups: Healthy controls (n=6, 6.19%), Depressive Syndrome (n=14, 14.43%), Subjective Cognitive Complaint (n=15, 15.46%), single-domain MCI (n=18, 18.56%), multiple-domain MCI (n=23, 23.71%), and Major Neurocognitive Disorder (n=21, 21.65%). Groups were comparable for sex (p=0.33) and most comorbidities but differed significantly in years of education (p=0.002), with the NCD group showing the lowest educational attainment (5.38 ± 3.07 years). Physical activity level also differed across groups (p=0.045), with the NCD group showing the highest proportion of sedentary patients (90%). Age showed a non-significant trend toward older values in NCD (79.9 ± 6.3 years, p=0.13).

Table 8. Study population across cognitive profiles.

	Total	Healthy	Depression	SCC	sMCI	mMCI	MND	p-value
	N=97	*N=6*	*N=14*	*N=15*	*N=18*	*N=23*	*N=21*	
Age	76.63 (6.99)	72.50 (6.63)	75.07 (6.72)	75.20 (5.67)	75.78 (10.61)	77.30 (3.81)	79.86 (6.26)	0.073
Sex	53 (55%)	3 (50%)	11 (79%)	9 (60%)	7 (39%)	11 (48%)	12 (57%)	0.332
Education (years)	7.98 (3.96)	11.00 (3.16)	8.21 (3.95)	9.00 (3.89)	9.33 (3.73)	7.70 (4.09)	5.38 (3.07)	**0.002**
Living place								0.688
Own house	95 (98%)	6 (100%)	14 (100%)	15 (100%)	18 (100%)	23 (100%)	19 (90%)	
Relatives' /friends' home	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	
Healthcare facility	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	
Marital status								0.066
Single	2 (2%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	

Married	56 (68%)	6 (100%)	7 (50%)	8 (80%)	13 (93%)	14 (67%)	8 (47%)	
Widow	22 (27%)	0 (0%)	4 (29%)	2 (20%)	1 (7%)	7 (33%)	8 (47%)	
Divorced	2 (2%)	0 (0%)	2 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Physical activity								**0.045**
No physical activity	40 (41%)	5 (83%)	2 (14%)	9 (60%)	8 (44%)	8 (35%)	8 (38%)	
<1 time/wk	34 (35%)	0 (0%)	9 (64%)	2 (13%)	4 (22%)	8 (35%)	11 (52%)	
1-2 times/wk	21 (22%)	1 (17%)	2 (14%)	3 (20%)	6 (33%)	7 (30%)	2 (10%)	
>3 times/wk	2 (2%)	0 (0%)	1 (7%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	
Alcohol consumption								0.058
No consumption	68 (70%)	4 (67%)	12 (86%)	11 (73%)	8 (44%)	14 (61%)	19 (90%)	
Occasional consumption	5 (5%)	0 (0%)	2 (14%)	1 (7%)	0 (0%)	2 (9%)	0 (0%)	
Former consumption	1 (1%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	
Current consumption	23 (24%)	2 (33%)	0 (0%)	3 (20%)	9 (50%)	7 (30%)	2 (10%)	
Smoking habit								0.430
Never	76 (78%)	6 (100%)	10 (71%)	11 (73%)	14 (78%)	20 (87%)	15 (71%)	
Former	13 (13%)	0 (0%)	1 (7%)	2 (13%)	2 (11%)	3 (13%)	5 (24%)	
Current	8 (8%)	0 (0%)	3 (21%)	2 (13%)	2 (11%)	0 (0%)	1 (5%)	
Body Mass Index (BMI)	26.68 (4.47)	25.53 (3.91)	27.29 (3.89)	25.93 (6.29)	25.82 (3.59)	27.34 (4.10)	27.16 (4.76)	0.679
Visit Reason								
Cognitive disturbances	88 (91%)	0 (0%)	13 (93%)	14 (93%)	18 (100%)	23 (100%)	20 (95%)	**<0.001**
Neuropsychiatric manifestations	34 (35%)	0 (0%)	5 (36%)	3 (20%)	6 (33%)	5 (22%)	15 (71%)	**0.002**
Mood disturbances	33 (34%)	0 (0%)	8 (57%)	6 (40%)	6 (33%)	4 (17%)	9 (43%)	0.069
Sleep disturbances	40 (41%)	0 (0%)	8 (57%)	6 (40%)	8 (44%)	4 (17%)	14 (67%)	**0.005**
Gait disturbances	4 (4%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	3 (14%)	0.132
Tremor	2 (2%)	0 (0%)	1 (7%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0.441
Functional changes	6 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (29%)	**<0.001**
Recurrent falls	10 (10%)	0 (0%)	3 (21%)	1 (7%)	0 (0%)	3 (13%)	3 (14%)	0.370
Mobility inside the home								**<0.001**
Indipendent	79 (81%)	6 (100%)	12 (86%)	14 (93%)	16 (89%)	22 (96%)	9 (43%)	
Independent with assistive device	13 (13%)	0 (0%)	2 (14%)	1 (7%)	2 (11%)	1 (4%)	7 (33%)	
With assistive device and supervision	5 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (24%)	
No. of chronic diseases	6.12 (2.83)	5.17 (1.83)	6.71 (2.46)	6.00 (3.16)	4.61 (2.68)	6.13 (2.96)	7.38 (2.56)	**0.032**
No. of drugs	6.44 (3.59)	3.83 (1.60)	8.79 (3.62)	6.53 (3.34)	4.00 (2.66)	6.78 (3.27)	7.29 (3.86)	**0.002**
Cardiovascular disease	1.26 (0.96)	1.00 (0.00)	1.21 (0.80)	1.40 (1.06)	1.00 (0.97)	1.30 (1.06)	1.43 (1.03)	0.746
Atrial fibrillation	13 (13%)	0 (0%)	2 (14%)	1 (7%)	3 (17%)	5 (22%)	2 (10%)	0.648
Chronic ischemic heart disease	11 (11%)	0 (0%)	2 (14%)	3 (20%)	0 (0%)	5 (22%)	1 (5%)	0.174
Chronic heart failure	5 (5%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	4 (19%)	**0.043**
Cardiometabolic disease	2.85 (1.64)	3.33 (1.51)	3.29 (1.59)	3.00 (1.69)	1.94 (1.06)	2.70 (1.79)	3.24 (1.73)	0.518
Arterial hypertension	75 (77%)	6 (100%)	11 (79%)	12 (80%)	12 (67%)	18 (78%)	16 (76%)	0.696
Dyslipidemia	52 (54%)	5 (83%)	8 (57%)	8 (53%)	11 (61%)	9 (39%)	11 (52%)	0.472
Carotid atherosclerosis	32 (33%)	1 (17%)	5 (36%)	4 (27%)	5 (28%)	8 (35%)	9 (43%)	0.818
Type 2 diabetes mellitus	28 (29%)	1 (17%)	6 (43%)	4 (27%)	3 (17%)	5 (22%)	9 (43%)	0.339
Overweight	30 (31%)	3 (50%)	6 (43%)	3 (20%)	4 (22%)	8 (35%)	6 (29%)	0.601
Obesity	16 (16%)	1 (17%)	2 (14%)	4 (27%)	2 (11%)	4 (17%)	3 (14%)	0.896
Hepatic steatosis	11 (11%)	1 (17%)	2 (14%)	2 (13%)	2 (11%)	1 (4%)	3 (14%)	0.895
Hypothyroidism	12 (12%)	1 (17%)	2 (14%)	3 (20%)	1 (6%)	3 (13%)	2 (10%)	0.864
Hyperthyroidism	1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0.600
Osteoporosis	7 (7%)	2 (33%)	2 (14%)	1 (7%)	0 (0%)	1 (4%)	1 (5%)	0.108
Chronic kidney disease	15 (15%)	0 (0%)	3 (21%)	1 (7%)	0 (0%)	4 (17%)	7 (33%)	0.054

Vascular leukoencephalopathy	34 (35%)	1 (17%)	2 (14%)	6 (40%)	6 (33%)	10 (43%)	9 (43%)	0.415
Stroke	6 (6%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)	2 (9%)	3 (14%)	0.400
Transient ischemic attack	4 (4%)	0 (0%)	1 (7%)	2 (13%)	0 (0%)	0 (0%)	1 (5%)	0.349
Hearing impairment	22 (23%)	0 (0%)	3 (21%)	3 (20%)	4 (22%)	4 (17%)	8 (38%)	0.410
Visual impairment	57 (59%)	1 (17%)	9 (64%)	9 (60%)	8 (44%)	17 (74%)	13 (62%)	0.137

Data presented as mean (SD) for continuous variables and n (%) for categorical variables. Continuous variables: Kruskal-Wallis; categorical variables: Chi-quadro / Fisher's test.
Abbreviations: SCC, Subjective Cognitive Complain; sMCI, Mild Cognitive Impairment – single domain; mMCI, Mild Cognitive Impairment – multiple domain; NCD, Neurocognitive Disorder; wk, week; No., number.

All 13 neuropsychological and functional measures differed significantly across the five diagnostic groups (Kruskal-Wallis, all $p<0.0033$ after Bonferroni correction for 15 variables), with the exception of TMT-B ($p=0.030$, nominally significant but not surviving correction). The strongest global differences were observed for MMSE ($p<0.001$) and CDT ($p<0.001$).

ROC analysis identified a pattern of test-specific discriminability across the five pairwise comparisons (Table 9). For early-stage comparisons (SCC vs sMCI, SCC vs mMCI), memory tests showed the highest discriminative performance: Immediate Recall achieved AUC=0.819 (95% CI: 0.672–0.965, $p<0.001$) for SCC vs sMCI, and Differential Recall AUC=0.803 (95% CI: 0.663–0.943, $p<0.001$) for SCC vs mMCI, while Matrix reached AUC=0.858 (95% CI: 0.737–0.979, $p<0.001$) for SCC vs mMCI. For the mMCI vs NCD comparison, global cognitive tests were most informative: MMSE AUC=0.913 (95% CI: 0.833–0.993, $p<0.001$) and CDT AUC=0.842 (95% CI: 0.732–0.951, $p<0.001$). As expected, the GDS-sf showed the highest discriminability for comparisons involving Depressive Syndrome, reflecting its construct validity as a mood assessment instrument: AUC=0.955 (95% CI: 0.902–1.000, $p<0.001$) for Depression vs mMCI and AUC=0.866 (95% CI: 0.738–0.993, $p<0.001$) for Depression vs NCD.

Table 9. Domain 1 - ROC Analysis for Neuropsychological Tests (adjusted) across cognitive profiles.

Domain 1	**Main Cognitive Domains**	**Comparison**				
		SCC vs sMCI	**SCC vs mMCI**	**mMCI vs NCD**	**Depr. vs mMCI**	**Depr. vs NCD**
MMSE	*Global*	0.839 (0.704-0.974) ***	0.794 (0.645-0.944) ***	0.913 (0.833-0.993) ***	0.658 (0.457-0.860)	0.925 (0.837-1.000) ***
Babcock	*Memory*	0.737 (0.568-0.906) **	0.758 (0.602-0.914) **	0.744 (0.597-0.892) **	0.509 (0.306-0.713)	0.704 (0.515-0.893) *
Immediate Recall	*Mem/Att*	0.819 (0.672-0.965) ***	0.810 (0.675-0.945) ***	0.687 (0.529-0.845) *	0.677 (0.492-0.862)	0.852 (0.699-1.000) ***

Differential Recall	*Memory*	0.809 (0.666-0.952) ***	0.803 (0.663-0.943) ***	0.609 (0.441-0.777)	0.750 (0.591-0.909) **	0.867 (0.740-0.995) ***
FAB	*Executive*	0.587 (0.389-0.785)	0.604 (0.415-0.793)	0.727 (0.573-0.880) **	0.540 (0.340-0.740)	0.748 (0.585-0.911) **
Matrix	*Att/Exec*	0.633 (0.437-0.829)	0.858 (0.737-0.979) ***	0.507 (0.327-0.687)	0.791 (0.628-0.954) ***	0.786 (0.625-0.946) ***
Maze	*Executive*	0.522 (0.423-0.621)	0.554 (0.451-0.656)	0.770 (0.643-0.897) ***	0.510 (0.381-0.639)	0.780 (0.638-0.922) ***
TMT-B	*Executive*	0.602 (0.400-0.803)	0.759 (0.588-0.931) **	0.514 (0.344-0.685)	0.623 (0.421-0.825)	0.614 (0.418-0.810)
TMT-A	*Attention*	0.539 (0.335-0.743)	0.675 (0.501-0.849) *	0.704 (0.549-0.858) **	0.562 (0.373-0.751)	0.741 (0.579-0.904) **
Fluency	*Language*	0.680 (0.494-0.865)	0.729 (0.569-0.889) **	0.728 (0.579-0.877) **	0.654 (0.460-0.847)	0.823 (0.675-0.971) ***
Praxia	*Perception*	0.559 (0.356-0.762)	0.567 (0.375-0.758)	0.815 (0.689-0.940) ***	0.517 (0.320-0.713)	0.817 (0.670-0.964) ***
CDT	*Executive*	0.563 (0.374-0.751)	0.661 (0.496-0.826)	0.842 (0.732-0.951) ***	0.596 (0.412-0.781)	0.918 (0.819-1.000) ***
GDS-sf	*Mood*	0.546 (0.341-0.752)	0.581 (0.402-0.760)	0.567 (0.391-0.743)	0.955 (0.902-1.000) ***	0.866 (0.738-0.993) ***

*AUC values with 95% confidence intervals from ROC. Comparisons between diagnostic groups were made using DeLong's method (DeLong) for p-value calculation (*p < 0.05, **p < 0.01, ***p < 0.001).*
Abbreviations: SCC, Subjective Cognitive Complain; sMCI, Mild Cognitive Impairment – single domain; mMCI, Mild Cognitive Impairment – multiple domain; NCD, Neurocognitive Disorder; MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery; TMT, Trail Making Test; CDT, Clock Drawing Test; GDS-sf, Geriatric depression scale short form.

Among domain 2 measures, handgrip strength was the only variable showing significant group differences with maximum handgrip strength ($p<0.001$), and handgrip residual (adjusted for sex and skeletal muscle mass) ($p<0.001$) both differed significantly across groups. Appendicular Skeletal Muscle Index (ASMI, $p=0.145$), fat mass, fat-free mass, and phase angle did not reach significance. Post-hoc analysis revealed that the NCD group had significantly lower handgrip residual compared to Depression ($p<0.01$) and sMCI ($p<0.01$), while mMCI also differed from Depression ($p<0.05$) suggesting that functional muscle weakness, independent of body size, is a feature of more advanced neurocognitive categories.

In ROC analysis, handgrip residual demonstrated meaningful discriminative performance particularly for comparisons involving more advanced neurocognitive categories, with AUC values reaching 0.749 ($p<0.001$) for mMCI vs NCD and 0.773 ($p<0.01$) for Depression vs mMCI, while performance was more modest for early-stage comparisons (SCC vs sMCI: AUC=0.652; SCC vs mMCI: AUC=0.516). These findings suggest that sex- and muscle mass-adjusted muscle strength contributes to diagnostic discrimination primarily at more advanced stages of the neurocognitive continuum, consistent with the established association between sarcopenia and dementia risk in older adults.

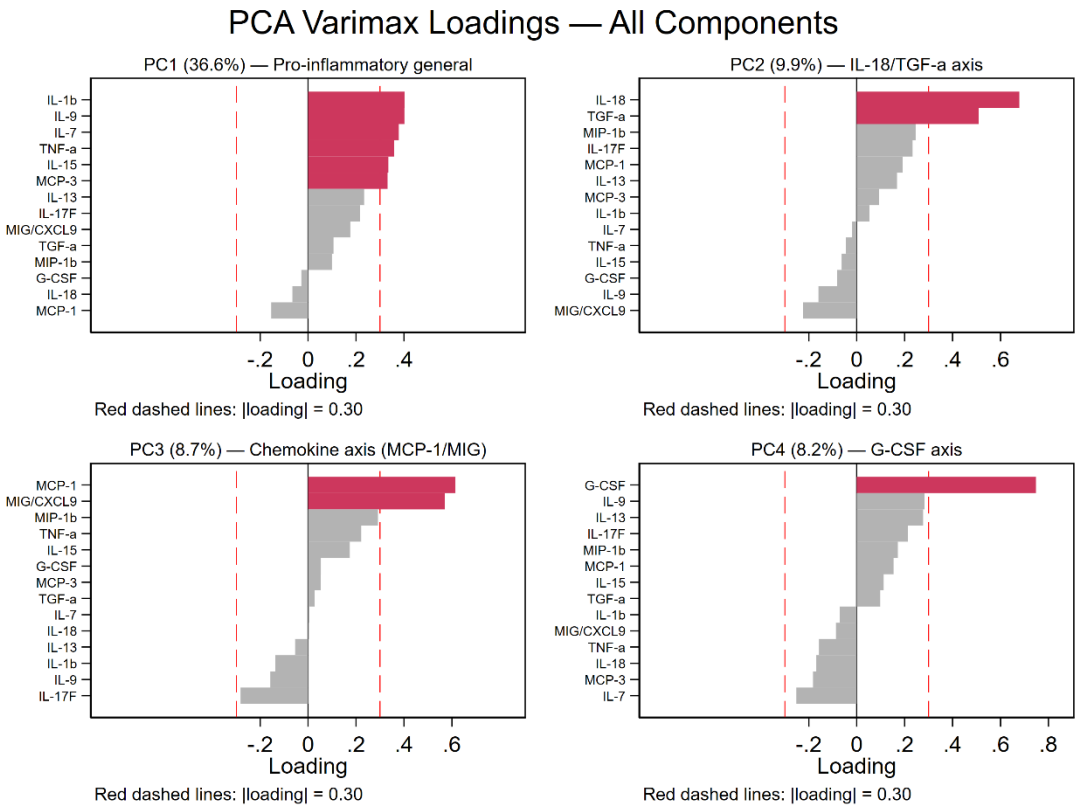
Of 27 metabolic and hematological variables examined, standard cardiometabolic indices including fasting glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, eGFR, and creatinine did not differ significantly across groups. Significant differences were observed for hepatic and inflammatory markers: AST ($p=0.004$), serum folate ($p=0.004$), white blood cell count ($p=0.012$), neutrophils ($p=0.017$), NLR ($p=0.017$), and lymphocytes ($p=0.049$). These findings suggest that low-grade hepatic inflammation and folate deficiency, rather than classical metabolic dysregulation, characterize the metabolic profile of patients with more advanced cognitive impairment.

In ROC analysis, serum folate emerged as the strongest metabolic discriminator, achieving AUC=0.839 ($p<0.001$) for SCC vs sMCI and AUC=0.796 ($p<0.001$) for mMCI vs NCD, while AST showed the highest performance for the mMCI vs NCD comparison (AUC=0.796, $p<0.001$).

Among the 45 plasma cytokines analyzed, 14 showed significant global group differences: TNF-α ($p<0.001$), IL-1β ($p<0.001$), IL-15 ($p<0.001$), IL-13 ($p=0.005$), TGF-α ($p=0.004$), MCP-3 ($p=0.006$), MIP-1β ($p=0.007$), IL-17F ($p=0.015$), MCP-1 ($p=0.021$), MIG/CXCL9 ($p=0.024$), IL-7 ($p=0.038$), IL-9 ($p=0.039$), IL-18 ($p=0.035$), and G-CSF ($p=0.027$).

Principal component analysis with varimax rotation applied to the 14 significant cytokines yielded four interpretable components explaining 63.4% of total variance (Figure 15). PC1 (36.6%) loaded on the pro-inflammatory general axis (IL-1β, IL-9, IL-13, TNF-α, IL-15, MCP-3). PC2 (9.9%) reflected the IL-18/TGF-α axis. PC3 (8.7%) captured the chemokine axis (MCP-1, MIG/CXCL9). PC4 (8.2%) was dominated by G-CSF. PC1 and PC3 scores showed the highest discriminative performance in ROC analysis across all five comparisons, with PC1 reaching AUC=0.891 ($p<0.001$) for Depression vs NCD and AUC=0.772 ($p<0.001$) for mMCI vs NCD, and PC3 achieving AUC=0.748 ($p<0.01$) and AUC=0.791 ($p<0.001$) for SCC vs sMCI and SCC vs mMCI respectively, suggesting a stage-dependent inflammatory signature.

Figure 15. Domain 4. Principal Component Analysis varimax loadings.



Each panel displays the varimax-rotated loadings of the 14 Kruskal-Wallis-significant cytokines (log-transformed) on each principal component. Bars highlighted in dark red indicate loadings exceeding the |0.30| threshold (red dashed lines). Variance: PC1 (36.6%); PC2 (9.9%); PC3 (8.7%); PC4 (8.2%). Cytokines are ordered by absolute loading magnitude within each component.

The progressive multidimensional model demonstrated consistent and substantial gains in discriminative performance with the sequential addition of each biological domain (Table 10, Figure 16). The demographic baseline model (M1) achieved modest AUC values ranging from 0.600 (SCC vs sMCI) to 0.777 (Depression vs NCD). The addition of the best-performing neuropsychological test (M2) produced the largest single incremental gain across all comparisons (ΔAUC range: +0.155 to +0.258), yielding AUC values between 0.773 and 0.966. Incorporating handgrip residual (M3) provided additional discriminative gain, most notable for the mMCI vs NCD comparison (ΔAUC=+0.039, AUC=0.973) and SCC vs sMCI (ΔAUC=+0.063, AUC=0.835). The metabolic component (M4) was applicable to two comparisons only serum folate z-score for SCC vs sMCI (ΔAUC=+0.027, AUC=0.863) and HbA1c z-score for SCC vs mMCI (ΔAUC=+0.012, AUC=0.899), and was omitted for the remaining three comparisons due to quasi-complete separation.

The inflammatory cytokine component (M5) provided the final incremental gain. In line with the stage-dependent inflammatory pattern identified in Domain 4, PC3 (chemokine axis) was selected for early-stage comparisons (SCC vs sMCI: ΔAUC=+0.094, final AUC=0.957; SCC vs mMCI: ΔAUC=+0.093, final AUC=0.922), while PC1 (pro-inflammatory axis) was selected for advanced-stage comparisons (mMCI vs NCD: ΔAUC=+0.015, AUC=0.988; Depression vs mMCI: ΔAUC=+0.013, AUC=0.997; Depression vs NCD: ΔAUC=+0.003, AUC=0.939).

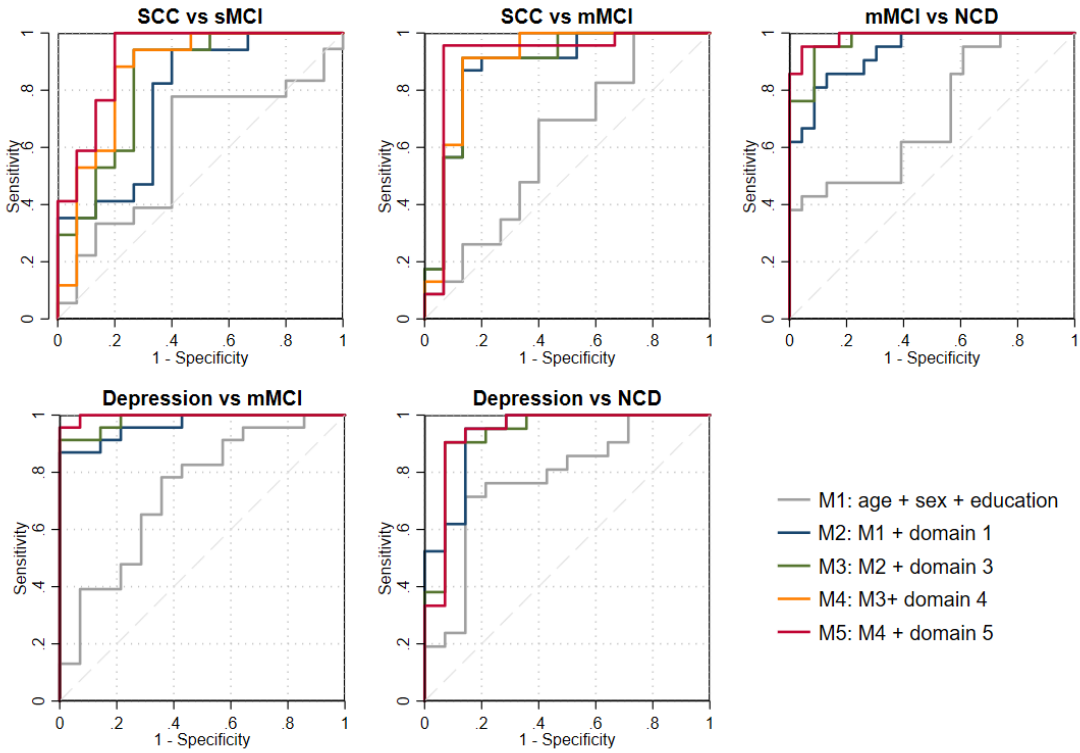
Bootstrap internal validation confirmed negligible optimism across all five comparisons (corrected AUC ≈ apparent AUC in all cases), with all replications successfully converging, suggesting that the high discriminative performance of the M5 models is not attributable to overfitting within the training sample.

Table 10. Progressive multidimensional predictive model: AUC and incremental discriminative gain (ΔAUC) across five pairwise diagnostic comparisons.

Comparison (N)	M1	M2 – Domain 1			M3 – Domain 2			M4 – Domain 3			M5 – Domain 4		
	AUC	AUC	Δ AUC	Variable	AUC	Δ AUC	Variable	AUC	Δ AUC	Variable	AUC	Δ AUC	Variable
SCC *(n=15)* **vs sMCI** *(n=18)*	0.600	0.773	**+0.173**	Imm. Recall	0.835	**+0.063**	Handgrip resid.	0.863	**+0.027**	Serum folate	0.957	**+0.094**	PC3
SCC *(n=15)* **vs mMCI** *(n=23)*	0.620	0.878	**+0.258**	Matrix	0.887	**+0.009**	Handgrip resid.	0.899	**+0.012**	HbA1c	0.922	**+0.093**	PC3
mMCI *(n=23)* **vs NCD** *(n=21)*	0.708	0.934	**+0.226**	MMSE	0.973	**+0.039**	Handgrip resid.	-	-	-	0.988	**+0.015**	PC1
Depr. *(n=14)* **vs mMCI** *(n=23)*	0.733	0.966	**+0.233**	GDS-sf	0.984	**+0.019**	Handgrip resid.	-	-	-	0.997	**0.013**	PC1
Depr. *(n=14)* **vs NCD** *(n=21)*	0.777	0.932	**+0.155**	MMSE	0.935	**+0.003**	Handgrip resid.	-	-	-	0.939	**+0.003**	PC1

Models estimated by Firth's penalized logistic regression. M1: age, sex, education. M2–M5: stepwise addition of the best-performing variable from each domain raw neuropsychological tests (Domain 1), body composition/strength (Domain 2), metabolic (Domain 3), inflammatory cytokine principal component (Domain 4). Dash indicates M4 was omitted due to quasi-complete separation.
Abbreviations: AUC, area under the ROC curve; NCD, Neurocognitive Disorder; SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains; MMSE, Mini Mental State Examination; GDS-sf, Geriatric Depression Scale short form; HbA1c, glycated hemoglobin; PC, Principal Component.

Figure 16. ROC curves. Multidimensional Predictive Model. Progressive model building across domains.



Each panel displays receiver operating characteristic curves for models M1 through M5 fitted by Firth's penalized logistic regression. M1 (grey): demographic baseline (age, sex, education). M2 (blue): M1 + best neuropsychological test. M3 (green): M2 + handgrip residual. M4 (orange): M3 + best metabolic predictor. M5 (red): M4 + best cytokine principal component. M4 was omitted for mMCI vs NCD, Depression vs mMCI, and Depression vs NCD comparisons due to quasi-complete separation. SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains; NCD, Neurocognitive Disorder.

To identify naturally occurring patient subgroups within the multidomain biological-cognitive space, hierarchical cluster analysis was applied to the same ten z-standardized variables used in the progressive predictive model. Two-cluster solution was identified as optimal, supported by the highest Calinski-Harabasz pseudo-F statistic (pseudo-F=27.5 for k=2, declining monotonically to 15.4 for k=5). The two clusters were approximately balanced in size (Cluster 1: n=49, 54%; Cluster 2: n=41, 46%) and differed consistently across all four biological domains (Table 11, Figure 17).

Cluster 1 was characterised by better global cognitive performance (mean MMSE=27.1 vs 19.6), better episodic memory (mean Differential Recall=8.9 vs 3.9), better executive-visuospatial function (mean CDT=8.8 vs 4.5), lower pro-inflammatory burden (mean cytokine PC1=−0.52 vs +1.27), higher serum folate (6.4 vs 5.1 ng/mL, p=ns), and better muscle strength (mean handgrip residual=+0.20 vs −1.02 kg), defining a relatively preserved cognitive-biological phenotype. Cluster 2 showed worse

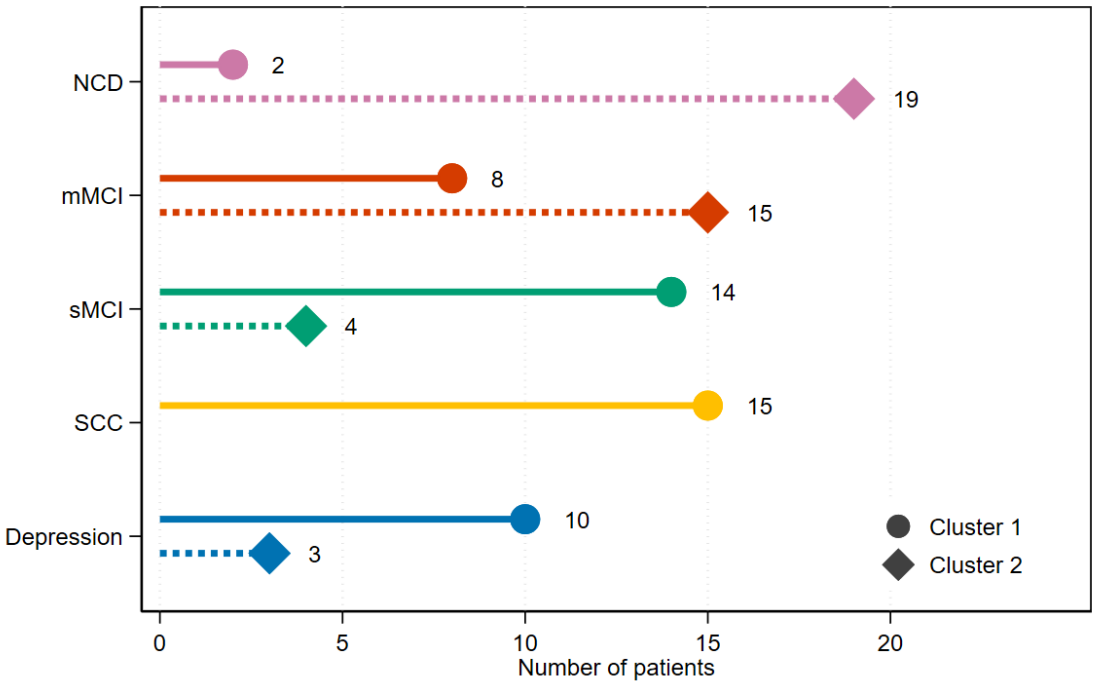
performance across all domains, consistent with a more advanced neurocognitive-inflammatory profile. Notably, metabolic variables did not significantly differ between clusters, suggesting that the biological separation is primarily driven by cognitive and inflammatory rather than metabolic factors. Examination of diagnostic group overlap confirmed the clinical coherence of this data-driven solution (Figure 17). NCD patients were almost exclusively assigned to Cluster 2 (19/21, 90%), while SCC patients were entirely in Cluster 1 (15/15, 100%). mMCI patients were distributed across both clusters (Cluster 1: n=8, Cluster 2: n=15), reflecting their intermediate biological position along the neurocognitive continuum. The Depressive Syndrome group showed a predominantly Cluster 1 distribution (10/13 with complete data, 77%), consistent with relatively preserved cognitive scores despite elevated mood burden and inflammatory markers.

Table 11. Multidomain biological-cognitive profile by cluster.

	Cluster 1 (*n=49*)	**Cluster 2 (*n=41*)**	**p-value**
Domain 1			
MMSE	27.1 ± 2.5	19.6 ± 6.2	<0.001
Differential Recall	8.9 ± 3.2	3.9 ± 3.5	<0.001
Fluency	15.2 ± 3.6	10.8 ± 4.0	<0.001
CDT	8.8 ± 1.5	4.5 ± 3.1	<0.001
Domain 2			
Handgrip residual	+0.20 ± 5.7	−1.02 ± 7.1	<0.05
Domain 3			
Serum folate	6.4 ± 3.4	5.1 ± 2.5	ns
Domain 4			
PC1	−0.52 ± 1.7	+1.27 ± 1.3	<0.001
PC3	−0.43 ± 1.2	+0.61 ± 1.1	<0.001

Mean ± standard deviation. Group differences assessed by Kruskal-Wallis test.
Abbreviations: MMSE, Mini Mental State Examination; CDT, Clock Drawing Test; PC, Principal Component; ns, not significant.

Figure 17. Multidomain cluster composition by diagnostic group.

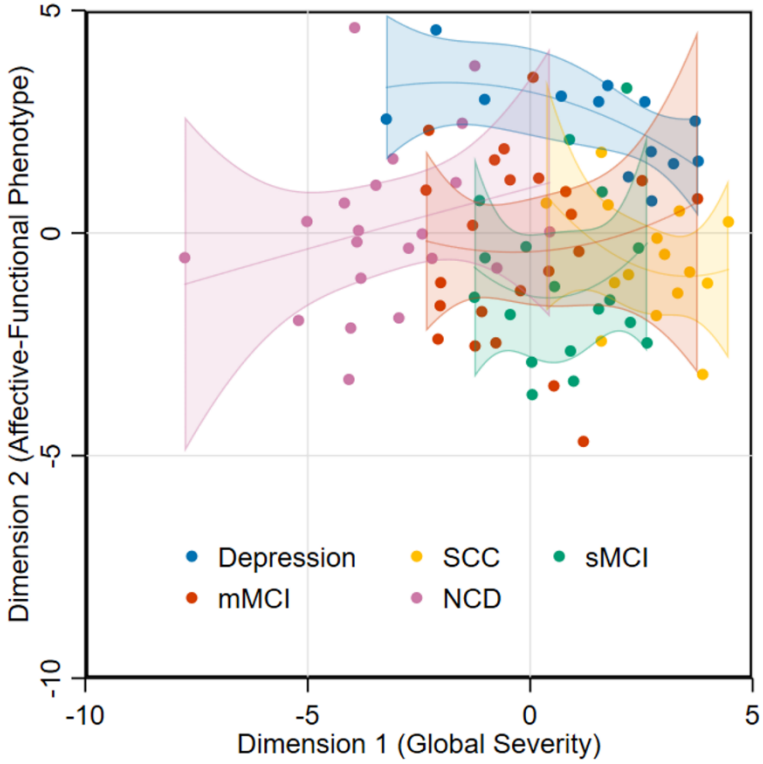


Each lollipop represents the number of patients per diagnostic group assigned to each cluster. Solid line/circle = Cluster 1 (n=49); dashed line/diamond = Cluster 2 (n=41). SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains; NCD, Neurocognitive Disorder.

To complement the discrete subgrouping obtained from cluster analysis, multidimensional scaling was applied to the same ten multidomain variables with the aim of deriving a spatially interpretable two-dimensional representation of patient heterogeneity. As shown in Figure 18, diagnostic groups occupied partially distinct regions of the bio-cognitive space, with a gradient of severity visible along the horizontal axis. The Depressive Syndrome group (blue) clustered in the upper-right quadrant, reflecting relatively preserved global cognition combined with prominent affective and functional burden. SCC (yellow) and sMCI (green) occupied central-to-right positions with higher Dimension 2 values, while mMCI (orange) and NCD (pink) shifted progressively toward lower values on both axes, consistent with greater cognitive-inflammatory impairment. The substantial overlap between groups, particularly in the central region, reflects the clinical continuum underlying these diagnostic categories and is consistent with the multidomain discriminative challenge addressed by the progressive predictive model. Dimension 1 (the Global Severity Index) was strongly correlated with all cognitive variables (MMSE: $\rho=0.874$, $p<0.001$; CDT: $\rho=0.747$, $p<0.001$; Differential Recall: $\rho=0.673$, $p<0.001$)

and inversely associated with systemic inflammation (PC1: $\rho=-0.629$, $p<0.001$; PC3: $\rho=-0.499$, $p<0.001$), confirming that this axis captures the cognitive-inflammatory severity gradient from SCC to NCD. Dimension 2 (the Affective-Functional Phenotype) showed selective associations with depressive burden (GDS: $\rho=-0.650$, $p<0.001$) and muscle strength (handgrip residual: $\rho=-0.641$, $p<0.001$), with no significant correlations with any purely cognitive measure, confirming its specificity for the affective-functional dimension that distinguishes Depressive Syndrome from MCI categories. To further validate the clinical relevance of the MDS solution, an exploratory logistic model predicting NCD from the two MDS dimensions achieved good discriminative performance (Pseudo-$R^2=0.574$, $p<0.001$), with Dimension 1 emerging as the sole significant predictor (OR=0.26, $p<0.001$), confirming that the Global Severity axis is the primary biological substrate distinguishing NCD from less severe diagnostic categories.

Figure 18. Bio-cognitive Profile Space derived from multidimensional scaling (MDS) of ten domain-integrated variables.



MDS was applied to z-standardized variables from four biological domains: neuropsychological performance (MMSE, Differential Recall, Matrix, Verbal Fluency, CDT, GDS-sf-inverted), body composition/strength (handgrip residual), metabolic (serum folate), inflammatory cytokine components (PC1, PC3). Each point represents an individual patient (n=90). Shaded bands represent quadratic fit curves with 95% confidence intervals per diagnostic group.

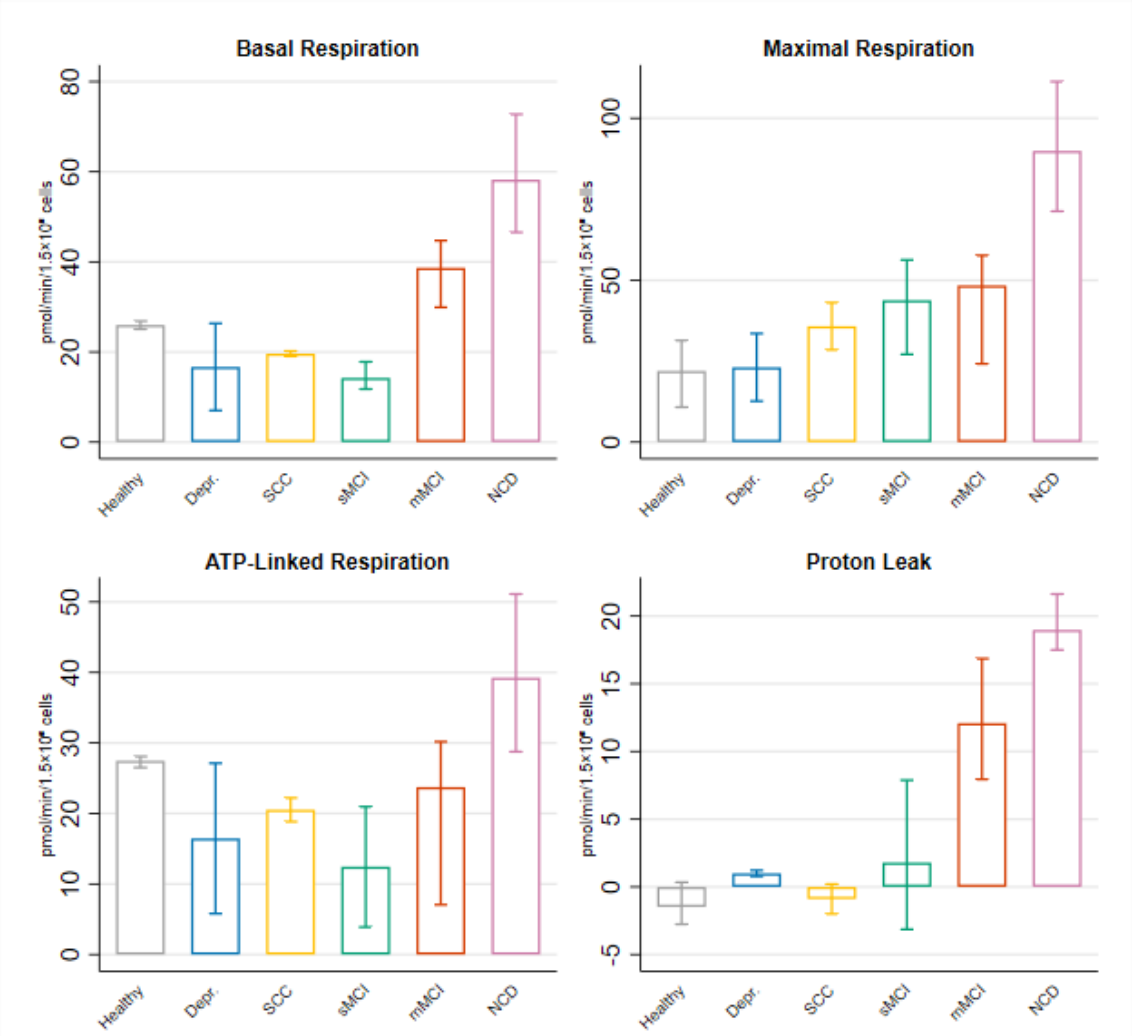
Abbreviations: NCD, Neurocognitive Disorder; SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains.

Seahorse XF metabolic profiling was available for a subsample of 18 patients across all six diagnostic groups (Healthy: n=3; Depression: n=2; SCC: n=2; sMCI: n=3; mMCI: n=5; NCD: n=3).

Mitochondrial respiration showed a descriptive pattern of progressive increase from early to advanced neurocognitive categories (Figure 19). Basal respiration was lowest in sMCI (14.3 ± 3.2 pmol/min) and highest in NCD (58.2 ± 13.3 pmol/min), with Healthy controls showing intermediate values (26.0 ± 0.9 pmol/min). A similar gradient was observed for maximal respiration (Healthy: 22.0 ± 10.4; sMCI: 43.8 ± 15.1; NCD: 89.9 ± 20.2 pmol/min) and ATP-linked respiration (Healthy: 27.5 ± 0.8; sMCI: 12.5 ± 8.5; NCD: 39.3 ± 11.2 pmol/min). Proton leak showed the most striking descriptive gradient, increasing from negative values in Healthy controls (−1.49 ± 1.62 pmol/min), consistent with tight mitochondrial coupling, to markedly elevated values in mMCI (12.1 ± 6.2 pmol/min) and NCD (19.0 ± 2.3 pmol/min), suggesting progressive uncoupling of mitochondrial respiration with advancing neurocognitive impairment. Depression and SCC showed intermediate proton leak values (1.00 ± 0.31 and −0.90 ± 1.56 pmol/min respectively).

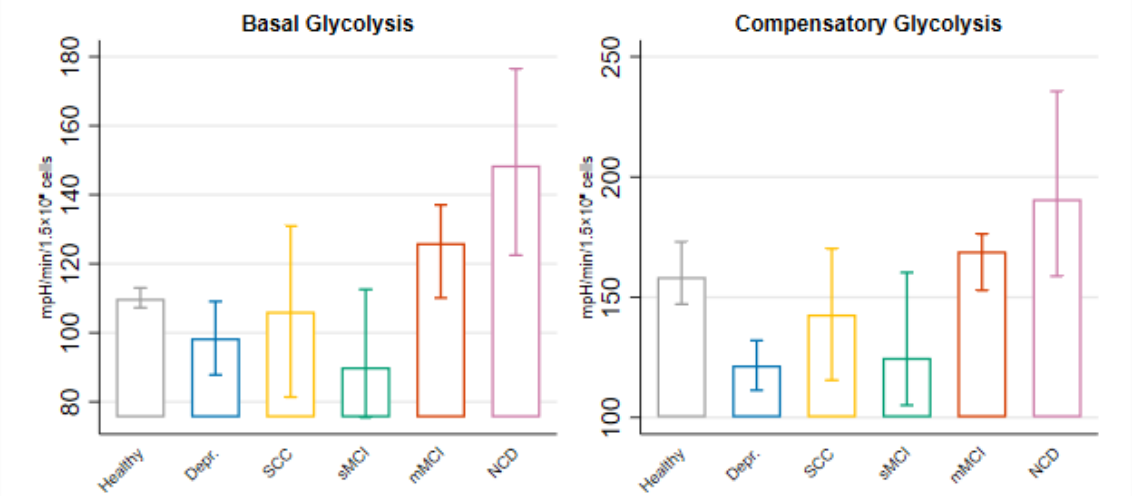
Glycolytic function showed a broadly similar descriptive pattern (Figure 20). Basal glycolysis was lowest in sMCI (90.0 ± 19.8 mpH/min) and highest in NCD (148.5 ± 27.1 mpH/min), with Healthy controls at 109.9 ± 2.9 mpH/min. Compensatory glycolysis followed the same trend (Healthy: 158.4 ± 13.3; sMCI: 124.8 ± 30.8; NCD: 190.8 ± 40.0 mpH/min), suggesting increased glycolytic demand in more advanced stages.

Figure 19. Mitochondrial respiration profile of peripheral blood mononuclear cells across diagnostic groups.



Data are presented as mean ± IQR given the non-parametric distribution; n per group: Healthy=3, Depression=2, SCC=2, sMCI=3, mMCI=5, NCD=3. Basal respiration reflects baseline oxygen consumption rate; maximal respiration reflects respiratory capacity under uncoupled conditions; ATP-linked respiration represents the fraction of basal respiration coupled to ATP synthesis; proton leak reflects mitochondrial uncoupling independent of ATP production.
Abbreviations: NCD, Neurocognitive Disorder; SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains.

Figure 20. Glycolytic function profile of peripheral blood mononuclear cells across diagnostic groups.



Data are presented as mean ± IQR; n per group: Healthy=3, Depression=2, SCC=2, sMCI=3, mMCI=5, NCD=3. Basal glycolysis reflects baseline extracellular acidification rate under physiological conditions; compensatory glycolysis reflects the maximal glycolytic capacity activated following mitochondrial inhibition.
Abbreviations: NCD, Neurocognitive Disorder; SCC, Subjective Cognitive Complaint; sMCI, Mild Cognitive Impairmente single domain; mMCI, Mild Cognitive Impairment multiple domains.

4.5 Discussion

Dementia represents a growing global challenge with profound consequences for patients, families, healthcare systems, and societies, compounded by increasing prevalence driven by demographic ageing and the marked heterogeneity of clinical presentations. Elderly patients with cognitive complaints rarely present in isolation: multimorbidity, polypharmacy, functional decline, and physical frailty are the rule rather than the exception, and all interact bidirectionally with cognitive trajectories through shared biological mechanisms, a complexity that demands a complex diagnostic approach. Against this background, we characterised a cohort of approximately one hundred geriatric outpatients spanning five diagnostic categories, from Subjective Cognitive Complaint to Major Neurocognitive Disorder, recruited in a geriatric memory clinic, and demonstrate that a multidimensional approach integrating neuropsychological performance, body composition, metabolic profile, and inflammatory cytokine profiling significantly improves diagnostic discrimination across clinically relevant pairwise comparisons along the neurocognitive continuum.

The five pairwise comparisons were pre-specified to reflect the most diagnostically challenging and clinically consequential boundaries along the neurocognitive continuum. The SCC vs sMCI and SCC

vs mMCI comparisons address the earliest diagnostic transition, the boundary between subjective complaint and early objectively documented cognitive decline, where single-domain models perform least well and preventive intervention has the greatest potential impact. The mMCI vs NCD comparison captures the critical threshold with direct implications for prognosis and treatment planning. The Depression vs mMCI and Depression vs NCD comparisons address the well-recognised diagnostic overlap between late-life depression and neurocognitive disorders, a distinction with profound therapeutic consequences.

Across all five comparisons, the demographic baseline model confirmed that age, sex, and education alone are insufficient for clinically meaningful stratification. The sequential integration of neuropsychological performance, body composition, metabolic markers, and inflammatory cytokine components produced progressive and consistent discriminative gains, an incremental structure with an important implication: the biological domains carry diagnostic information that is not redundant with neuropsychological performance. Each domain captures a distinct facet of the multidimensional pathophysiology of neurocognitive decline, functional muscle weakness, micronutrient and metabolism, and systemic inflammation, none of which is fully captured by cognitive testing alone. While the largest single incremental gain was produced by the neuropsychological step (ΔAUC: +0.155 to +0.258), the inflammatory cytokine component delivered the largest biological increment precisely for the most clinically difficult comparisons, SCC vs sMCI (ΔAUC=+0.094) and SCC vs mMCI (ΔAUC=+0.093), where cognitive tests alone are least discriminative. This finding suggests that early-stage inflammatory dysregulation, captured by the chemokine axis in peripheral blood, may represent a biological signature of the SCC-to-MCI transition that precedes or accompanies the earliest detectable cognitive changes.

The neuropsychological findings confirmed a pattern of test-specific discriminability consistent with the known cognitive phenotypes of each diagnostic category. Importantly, all scores were analysed as continuous variables rather than dichotomised at normative cut-offs, an approach that preserves the full gradient of performance variability and is more sensitive to subtle differences that categorical classification would obscure, particularly for early-stage comparisons where many patients score within the normal range by conventional criteria.

For early-stage comparisons, episodic memory tests showed the highest discriminative performance, reflecting the primacy of encoding and delayed retrieval deficits in early MCI. For comparisons involving NCD, global and visuospatial-executive tests dominated, consistent with the pervasive nature of cognitive impairment at this stage. As expected, the GDS-sf was the most discriminative instrument for comparisons involving Depressive Syndrome, confirming that the affective-cognitive overlap can be partially resolved at the neuropsychological level even before biological profiling. Notably, no single test achieved consistently high performance across all five comparisons, reinforcing the rationale for selecting the best-performing test per comparison in the progressive predictive model. Among all body composition parameters, only the sex- and muscle mass-adjusted handgrip residual showed significant group differences and meaningful discriminative performance. Appendicular Skeletal Muscle Index, fat mass, fat-free mass, and phase angle did not differ across diagnostic groups. This dissociation between functional muscle capacity and structural measures is consistent with the concept of functional sarcopenia [100], where the loss of muscle strength precedes or exceeds the loss of muscle mass and carries greater prognostic relevance for adverse outcomes including cognitive decline [104]. Analysed as a continuous variable, the handgrip residual revealed a graded reduction across the neurocognitive spectrum that categorical sarcopenia classification would have partially obscured. Its incremental contribution was small but consistent across comparisons (ΔAUC up to +0.063 for SCC vs sMCI), suggesting that functional muscle weakness captures biological information complementary to neuropsychological performance, and that in this population it is the functional rather than the compositional dimension of muscle that is relevant to the neurocognitive trajectory.

Standard cardiometabolic indices showed no discriminative gradient across the five comparisons, suggesting that classical metabolic dysregulation, while a recognised longitudinal risk factor for dementia, does not differentiate diagnostic categories at a single cross-sectional assessment in a clinically managed outpatient cohort. In contrast, serum folate and AST emerged as the most informative metabolic discriminators, pointing to micronutrient status and subclinical hepatic inflammation as the biologically relevant metabolic dimension in this population. Low serum folate, a risk factor for cognitive decline through hyperhomocysteinemia and impaired one-carbon metabolism [105], showed its highest discriminative performance for SCC vs sMCI, suggesting that

folate insufficiency may be a modifiable biological contributor to the earliest stages of objective impairment . Elevated AST reflects the low-grade systemic inflammatory activity, inflammaging, mechanistically linked to neurodegeneration [106], consistent with the inflammatory findings of Domain 4. These findings suggest that metabolic profiling in this context should extend beyond the standard cardiometabolic panel to include micronutrient assessment and inflammatory haematological indices.

Among the 45 plasma cytokines analysed, 14 showed significant global group differences, spanning pro-inflammatory interleukins, chemokines, and regulatory cytokines, a broad pattern consistent with the systemic and multifaceted nature of inflammatory dysregulation in neurocognitive decline. PCA of these 14 cytokines identified four inflammatory axes, of which two showed distinct stage-dependent discriminative profiles: PC1 (pro-inflammatory general axis: IL-1β, TNF-α, IL-15) was most informative for advanced-stage comparisons, while PC3 (chemokine axis: MCP-1, MIG/CXCL9) was most informative for early-stage comparisons. The dimensionality reduction achieved by PCA is methodologically important, allowing the inflammatory signal to be incorporated into the predictive model parsimoniously and avoiding overfitting in small-sample logistic models.

The stage-dependent pattern has important biological implications. The chemokine axis reflects early peripheral immune cell mobilisation and monocyte recruitment, consistent with the hypothesis that the earliest phase of neuroinflammation precedes the full cytokine storm. Elevated MCP-1 levels have been reported in MCI and mild AD, and higher CSF MCP-1 concentrations in prodromal AD have been associated with faster cognitive decline [107],[108]. By contrast, the generalised pro-inflammatory signature of PC1 dominates in advanced stages, reflecting the escalating neuroinflammatory cascade characteristic of established MCI and dementia, where chronic activation of IL-1β and TNF-α drives synaptic dysfunction and neuronal damage [109]. This biological differentiation suggests that inflammatory profiling should not be reduced to a single biomarker but mapped onto the stage of the disease trajectory.

To characterise the multidimensional structure of patient heterogeneity beyond pairwise comparisons, we applied two complementary unsupervised approaches to the same ten domain-integrated variables. MDS provides a continuous spatially interpretable representation of biological-cognitive variability,

while hierarchical cluster analysis yields a discrete partition into biologically homogeneous subgroups. Their convergence strengthens the interpretability and clinical relevance of both.

The two-dimensional bio-cognitive profile space captured the major axes of patient heterogeneity. Dimension 1, the Global Severity Index, reflects the cognitive-inflammatory gradient confirmed by strong correlations with MMSE, CDT, and cytokine PC1 whereas Dimension 2, the Affective-Functional Phenotype, captures depressive burden and physical function independently of global cognition, selectively associated with GDS and handgrip residual with no significant correlation with any purely cognitive measure. A key implication is the visualisation of the clinical continuum: diagnostic groups occupy overlapping regions of the bio-cognitive space, arguing against the assumption that categorical diagnosis fully captures underlying biological complexity.

Cluster analysis provided the discrete complement, identifying a two-cluster solution as optimal. The clusters mapped coherently onto the MDS structure: Cluster 1 (preserved phenotype) included all SCC patients (15/15, 100%) and most Depression patients (77%); Cluster 2 (impaired phenotype) captured nearly all NCD patients (19/21, 90%). The intermediate position of mMCI patients, distributed across both clusters, confirms the biological heterogeneity of this category and suggests that biological profiling may identify which patients carry an already-impaired phenotype consistent with imminent progression. The predominantly Cluster 1 distribution of the Depressive Syndrome group, consistent with its high Dimension 2 positioning, further supports the biological distinctiveness of late-life depression from neurocognitive disorders, a finding with direct clinical relevance for differential diagnosis and therapeutic decision-making.

The exploratory bioenergetic profiling of PBMCs (n=18) revealed a descriptive pattern of progressive metabolic dysregulation internally consistent with the Domain 4 inflammatory findings. The most notable observation concerns proton leak, which showed a marked gradient from near-zero values in Healthy controls, consistent with tight mitochondrial coupling, to progressively elevated values in mMCI and NCD, suggesting stage-dependent mitochondrial uncoupling. The elevated basal and maximal respiration in NCD is more plausibly interpretable as compensatory upregulation in response to oxidative stress than as preserved function, while the parallel increase in glycolysis points to a Warburg-like metabolic shift in chronically activated immune cells [110]. Notably, the patients showing

the highest pro-inflammatory cytokine burden (PC1) also displayed the most pronounced bioenergetic dysregulation, suggesting a feed-forward loop between mitochondrial uncoupling and systemic inflammation. These observations are strictly hypothesis-generating given the sample size, but if replicated, PBMC proton leak could represent a minimally invasive window into the systemic metabolic dysregulation accompanying neurocognitive progression.

The findings of this study have several translational implications. First, the multidomain model demonstrates that meaningful diagnostic discrimination is achievable with biologically accessible and clinically routine tools, blood tests for cytokines, metabolic markers, and folate; handgrip dynamometry; and bioimpedance, making the approach potentially scalable in geriatric outpatient settings. Second, the specific biological contributors identified at each diagnostic transition suggest domain-specific therapeutic targets: folate supplementation at the SCC-to-MCI boundary; anti-inflammatory interventions at the mMCI-to-NCD transition; and exercise-based interventions, particularly resistance training, across all comparisons given the consistent contribution of muscle strength. Third, the bio-cognitive profiling framework offers a conceptual tool for patient stratification beyond the categorical diagnosis: a patient positioned in the impaired cluster or the low Dimension 1 region of the MDS space may warrant more intensive monitoring and earlier intervention regardless of formal DSM-5 category, representing a step toward individualised care pathways in geriatric cognitive medicine.

Several limitations must be acknowledged. The cross-sectional design precludes causal inference and longitudinal trajectory assessment, future longitudinal studies will be required to determine whether the biological profiles identified here predict clinical progression. The sample was recruited in a single tertiary memory clinic, limiting generalisability to primary care or population-based settings. Small group sizes in several comparisons result in wide confidence intervals and borderline EPV ratios in some models; although bootstrap validation confirmed negligible internal optimism, external validation in an independent cohort is mandatory before clinical implementation. The bioenergetic analysis is strictly exploratory. The absence of neuroimaging and cerebrospinal fluid biomarkers prevents comparison with established biomarker-based diagnostic frameworks. Finally, the absence

of multiple testing correction for ROC and logistic analyses, methodologically justified by the hypothesis-driven design, should be considered when interpreting individual results.

This study provides empirical evidence that a multidimensional approach integrating neuropsychological, body composition, metabolic, and inflammatory cytokine profiling significantly improves diagnostic discrimination across the neurocognitive continuum in elderly outpatients. Each biological domain contributes independent incremental information beyond cognitive testing, and the integrated model achieves excellent discriminative performance across all five comparisons including the most diagnostically challenging early-stage boundaries. The bio-cognitive profile space reveals a biologically structured organisation of patient heterogeneity with two interpretable dimensions, while unsupervised clustering identifies two discrete phenotypes that map coherently onto clinical diagnosis. These findings support a paradigm shift toward comprehensive biological-cognitive characterisation of the elderly patient with cognitive complaints, a framework that is not only scientifically more accurate but clinically more actionable, enabling targeted interventions on modifiable biological determinants of the cognitive trajectory. External validation and longitudinal follow-up remain necessary before clinical implementation, but the multidomain framework presented here provides a rigorous methodological and biological foundation for the next steps in precision geriatric cognitive medicine.

5. STUDY 3 - INFLUENCE OF LIFESTYLE CHANGES AND BODY COMPOSITION ON NAFLD EVOLUTION IN A MULTICENTRE COHORT

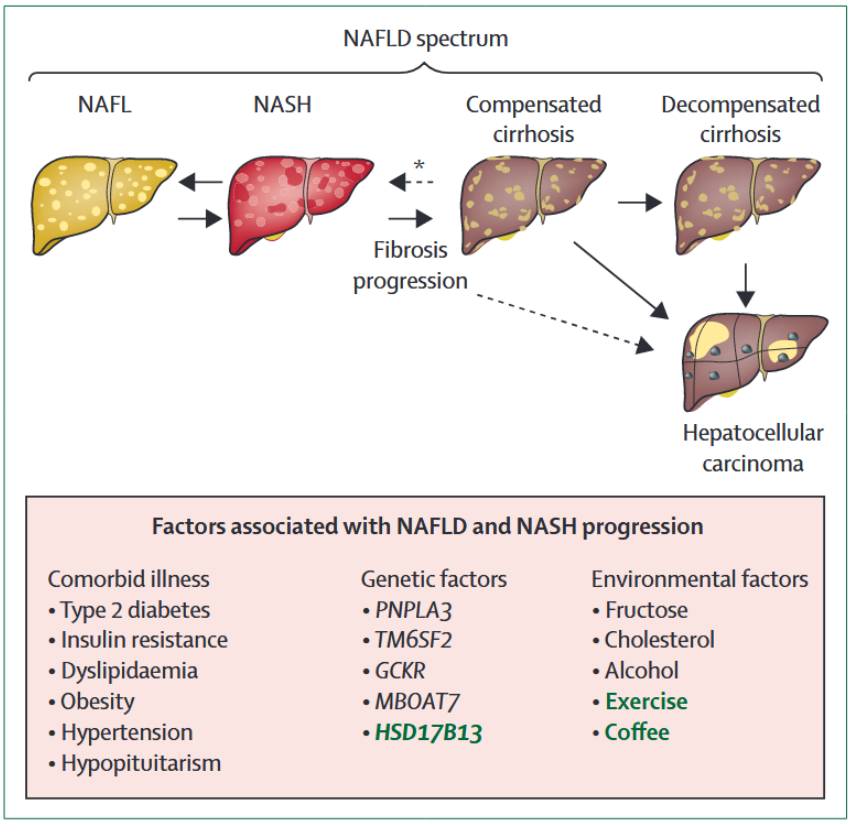
5.1 Background

Steatotic liver disease encompasses a heterogeneous group of conditions characterised by excessive hepatic fat accumulation arising from different aetiologies. Within this framework, metabolic dysfunction–associated steatotic liver disease (MASLD) has been formally introduced to reflect the central role of cardiometabolic dysfunction in the development of hepatic steatosis. According to the joint EASL–EASD–EASO Clinical Practice Guidelines, MASLD is defined by the presence of hepatic steatosis in conjunction with at least one cardiometabolic risk factor, including obesity, type 2 diabetes, dyslipidaemia, or hypertension, and in the absence of alternative causes of steatosis [111].

MASLD includes a wide histopathological and clinical spectrum, ranging from isolated steatosis (MASL) to metabolic dysfunction–associated steatohepatitis (MASH), which is characterised by hepatocellular ballooning and lobular inflammation, and further to progressive fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). This continuum mirrors the previously established NAFLD (Non-Alcoholic Fatty Liver Disease) – NASH (Nonalcoholic Steatohepatitis) spectrum, and extensive re-analyses of large cohorts demonstrate near-complete overlap between populations classified using NAFLD and MASLD criteria, supporting the transferability of historical NAFLD evidence to the MASLD framework [111]. Despite the nomenclature shift, NAFLD remains deeply embedded in the scientific literature, particularly in epidemiological and mechanistic studies (Figure 21).

NAFLD is traditionally defined as hepatic steatosis affecting more than 5% of hepatocytes, associated with metabolic risk factors, and occurring in the absence of excessive alcohol intake or other chronic liver diseases [112]. Histologically, NAFLD comprises non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), the latter representing the necroinflammatory and progressive form of the disease [112],[113]. Importantly, disease activity (steatosis and inflammation) and disease stage (fibrosis) are partially dissociable. While steatosis and inflammation may fluctuate over time, fibrosis severity is the strongest determinant of prognosis, liver-related outcomes, and mortality [111],[112].

Figure 21. Spectrum of NAFLD [112].



*Factors in black have an established association with NAFLD and NASH progression (broadly classified into comorbid illness, genetic factors, and environmental factors).34 Green indicates a protective factor. NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis. *Fibrosis regression.*

5.1.1 *Epidemiology of NAFLD*

Over the past four decades, NAFLD has emerged as the most prevalent chronic liver disease worldwide, paralleling global trends in obesity, insulin resistance, and type 2 diabetes [112],[114]. A large systematic review and meta-analysis indicates that global NAFLD prevalence increased from approximately 25.3% in 1990–2006 to 38.0% in 2016–2019, reflecting a more than 50% relative increase over three decades [114]. In line with these data, the EASL–EASD–EASO guidelines estimate that NAFLD now affects more than 30% of the general population, with prevalence expected to continue rising [111].

Prevalence is substantially higher in populations with cardiometabolic disease. NAFLD affects nearly half to two-thirds of individuals with type 2 diabetes and up to 80–90% of individuals with obesity, particularly severe obesity [112],[113]. These figures underscore the tight coupling between hepatic

steatosis and systemic metabolic dysfunction, reinforcing the concept of NAFLD as a hepatic manifestation of multisystem metabolic disease rather than an isolated liver disorder [113].

Although only a minority of individuals with NAFLD progress to advanced liver disease, the sheer magnitude of the affected population translates into a rapidly increasing absolute burden of cirrhosis, hepatic decompensation, and HCC [112],[114]. Modelling studies project substantial increases in NAFLD-related cirrhosis and liver-related mortality by 2030, driven by population ageing, longer duration of metabolic exposure, and rising obesity prevalence in younger cohorts [114]. The EASL–EASD–EASO guidelines similarly frame NAFLD as a major global public health challenge with profound socioeconomic implications [111].

As in other regions, the epidemiology of NAFLD in Europe is closely linked to the increasing prevalence of overweight, obesity, and type 2 diabetes; however, marked heterogeneity persists across countries, populations, and study designs. Recent meta-analyses estimate the overall prevalence of NAFLD in Europe to range between approximately 23.7% and 26.9%, with more recent analyses incorporating data up to 2019 suggesting a further increase to around 30.9%. The prevalence of NAFLD in Europe mirrors the distribution of underlying metabolic risk factors. According to Eurostat data, more than 50% of adults in the European Union were overweight in 2019, with marked variation between countries and between sexes. Similarly, the number of adults diagnosed with diabetes in the EU has nearly doubled over the past two decades, increasing from approximately 16.8 million in 2000 to over 32 million in 2019, a trend that is expected to further fuel the NAFLD epidemic [115]. In populations with obesity or type 2 diabetes, NAFLD prevalence substantially exceeds that observed in the general population, consistent with the close metabolic underpinnings of the disease [112]. The burden of NAFLD-related hepatocellular carcinoma is also rising in Europe, reflecting both the increasing prevalence of NAFLD and the declining contribution of viral hepatitis. European studies indicate that NAFLD accounted for 0–3% of HCC cases in the 1990s, increasing to 12–29% in the 2010s, with projections suggesting that fatty liver disease may already represent the leading aetiology of HCC in some countries, including Italy [112].

5.1.2 *Aetiology and risk factor of liver disease*

At the centre of NAFLD pathogenesis lies metabolic dysfunction, with obesity and type 2 diabetes representing the most influential risk factors for both disease onset and progression. The EASL–EASD–EASO guidelines emphasise that obesity, particularly visceral adiposity, and type 2 diabetes are the strongest predictors of advanced fibrosis, cirrhosis, and HCC [111]. Insulin resistance acts as a unifying mechanism linking adipose tissue dysfunction, dyslipidaemia, hepatic fat accumulation, and inflammatory activation [112],[113]. Visceral fat distribution is of particular relevance, as abdominal obesity mediates much of the cardiometabolic risk associated with NAFLD. Waist circumference, although an imperfect surrogate, correlates more closely with hepatic steatosis and fibrosis risk than body mass index alone.

Disease prevalence and severity increase with age, reflecting the long latency of NAFLD progression and cumulative exposure to metabolic stressors [114]. Post-menopausal status is associated with higher NAFLD prevalence and more advanced fibrosis, partly mediated by hormonal changes and redistribution of body fat toward a more visceral phenotype [111]. Importantly, earlier onset of obesity and metabolic dysfunction confers a longer duration of disease exposure, substantially increasing lifetime risk of cirrhosis and HCC [114].

Urbanisation, dietary westernisation, and sedentary behaviour are widely implicated in NAFLD epidemiology, although rising BMI in rural populations also contributes significantly [114]. Beyond total caloric excess, specific dietary components play mechanistic roles. Grander and colleagues highlight fructose as a key driver of hepatic de novo lipogenesis and disease progression, particularly through activation of lipogenic transcription factors and increased substrate availability for triglyceride synthesis [113]. Socioeconomic disparities further modulate exposure to obesogenic environments, diet quality, and physical activity, thereby influencing NAFLD risk at the population level [111].

NAFLD exhibits moderate heritability, with genetic factors accounting for a substantial proportion of interindividual variability in disease susceptibility and severity [112]. The PNPLA3 I148M variant is the most consistently associated genetic determinant, influencing hepatic lipid handling, inflammation, fibrosis, and HCC risk [112],[113]. However, genetic risk typically manifests in interaction

with environmental and metabolic exposures, particularly obesity, underscoring the importance of gene–environment interplay rather than deterministic genetic causation.

The NAFLD framework explicitly recognises MetALD, describing individuals with metabolic steatosis who consume moderate amounts of alcohol below classical thresholds for alcohol-related liver disease. MetALD is associated with worse prognosis and higher all-cause mortality, highlighting the synergistic toxicity of alcohol and metabolic dysfunction. Evidence consistently supports a supra-additive interaction between alcohol intake and obesity or insulin resistance, increasing risks of fibrosis progression and HCC, thereby complicating the establishment of "safe" alcohol thresholds in this population [111].

5.1.3 *Pathogenesis and mechanisms of liver disease*

NAFLD pathogenesis reflects a complex network of immunometabolic dysregulation, rather than a linear "two-hit" process. Overnutrition leads to expansion and dysfunction of adipose tissue, particularly visceral fat depots, which become infiltrated by macrophages and secrete pro-inflammatory cytokines that promote systemic insulin resistance [112]. Insulin resistance increases lipolysis and free fatty acid flux to the liver while simultaneously enhancing hepatic de novo lipogenesis, overwhelming hepatocellular metabolic capacity [113].

The resulting accumulation of toxic lipid species drives lipotoxicity, characterised by oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and activation of inflammatory signalling pathways, including NF-κB, JNK, and inflammasome complexes [112],[113]. Free cholesterol, in particular, has been implicated as a potent pro-inflammatory and pro-fibrotic mediator, promoting mitochondrial oxidative injury and sensitising hepatocytes to cytokine-induced damage [113].

Crosstalk between the liver, adipose tissue, and gut further amplifies disease progression. Alterations in gut microbiota composition, intestinal permeability, and bile acid signalling influence hepatic inflammation and metabolism, although causal pathways remain incompletely defined [112],[113].

Ultimately, fibrogenesis represents the final common pathway linking chronic metabolic injury to clinically relevant liver disease. Hepatic stellate cell activation, driven by inflammatory and profibrotic

mediators, leads to extracellular matrix deposition and architectural distortion, culminating in cirrhosis and its complications [111]–[113].

5.1.4 *Diagnosis of liver disease*

The diagnosis and risk stratification of NAFLD are based on a range of complementary diagnostic modalities, encompassing clinical evaluation, biochemical markers, and multiple imaging-based techniques, which are applied in a stepwise and context-dependent manner to balance diagnostic accuracy with feasibility and cost-effectiveness.

Abdominal ultrasonography remains the most widely used first-line imaging modality for the detection of hepatic steatosis in both primary care and specialist settings, owing to its broad availability, low cost, and non-invasive nature [111]. In routine clinical practice, ultrasonography is often the initial test that raises suspicion of NAFLD, particularly in individuals with metabolic risk factors or persistently elevated liver enzymes. On ultrasound, hepatic steatosis is typically identified by increased liver echogenicity ("bright liver"), blurring of intrahepatic vascular margins, and attenuation of the ultrasound beam in deeper portions of the liver. Ultrasonography has important limitations that constrain its role in diagnosis and risk stratification. Its sensitivity decreases substantially when hepatic fat content is mild, generally below 20–30%, and it cannot reliably quantify liver fat content or distinguish between simple steatosis and steatohepatitis [111],[116]. Moreover, ultrasound provides no direct information on the presence or stage of liver fibrosis, which is the strongest determinant of liver-related outcomes in NAFLD. Advanced fibrosis and cirrhosis may alter liver echotexture and vascular architecture, but these features are neither sensitive nor specific enough to allow accurate staging. Consequently, a normal ultrasound does not exclude clinically significant disease, and steatosis detected on ultrasound does not equate to increased liver-related risk in the absence of fibrosis [117].

Fibrosis alters the mechanical properties of liver tissue, enabling non-invasive assessment through elastography techniques. Vibration-controlled transient elastography (VCTE) is the most widely used method, providing measurements of liver stiffness (LSM) as a surrogate for fibrosis and controlled attenuation parameter (CAP) values to estimate steatosis. VCTE offers reasonable accuracy for fibrosis staging, although reliability decreases in individuals with severe obesity. Alternative

ultrasound-based techniques, such as point shear wave elastography and two-dimensional shear wave elastography, show comparable diagnostic performance.

Magnetic resonance elastography (MRE) provides at least equivalent, and in some studies superior, accuracy for staging advanced fibrosis, but its high cost and limited availability restrict widespread use.

Blood-based fibrosis scores combine routine laboratory parameters with demographic and anthropometric variables to estimate the likelihood of advanced fibrosis. These scores outperform isolated liver enzyme measurements, which have limited discriminatory power. The most widely used first-line test is the Fibrosis-4 index (FIB-4), calculated using age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count. FIB-4 is favoured because of its simplicity, widespread availability, and low cost [118]. However, its performance is limited in certain populations, particularly individuals with type 2 diabetes and older adults. In those aged over 65 years, a higher rule-out cut-off (2.0 instead of 1.3) is recommended to reduce false-positive results. The NAFLD fibrosis score (NFS) incorporates additional variables, including body mass index, albumin levels, and impaired fasting glucose or diabetes status [119]. Both FIB-4 and NFS demonstrate moderate accuracy for detecting advanced fibrosis (AUROCs approximately 0.75–0.77) and perform poorly in individuals younger than 35 years [120]. Their principal strength lies in their high negative predictive value, making them effective tools for ruling out advanced fibrosis in low-prevalence settings. Conversely, positive predictive value is modest, leading to a substantial proportion of false positives when these scores are used in isolation.

Despite advances in non-invasive diagnostics, liver biopsy remains the reference standard for assessing microscopic features such as hepatocyte ballooning, lobular inflammation, and definitive staging of fibrosis. However, because histological features of steatohepatitis do not consistently alter management decisions, liver biopsy is generally not required for routine clinical care in NAFLD. Its role is largely confined to clinical trials, diagnostic uncertainty, or exclusion of alternative liver diseases [111].

5.1.5 *Comorbidities associated with liver disease*

NAFLD is increasingly recognised as a systemic disease with multisystem consequences, extending far beyond the liver. Cardiovascular disease represents the leading cause of death in individuals with NAFLD, exceeding liver-related mortality in most non-cirrhotic populations. Although NAFLD shares many risk factors with cardiovascular disease, accumulating evidence suggests that NAFLD, particularly when associated with advanced fibrosis, may independently contribute to cardiovascular risk through mechanisms involving systemic inflammation, endothelial dysfunction, proatherogenic lipid profiles, and altered glucose metabolism [112]. Chronic kidney disease is another major comorbidity, with MASLD associated with increased risk of reduced glomerular filtration rate and albuminuria, independent of traditional risk factors [111]. The bidirectional relationship between NAFLD and type 2 diabetes is particularly strong: NAFLD often precedes diabetes onset, while diabetes markedly accelerates liver disease progression and increases HCC risk [111]–[113]. Chronic low-grade inflammation, insulin resistance, and adipokine dysregulation are hypothesised mediators linking hepatic steatosis to carcinogenesis, though causal pathways remain under investigation. Additional associations include obstructive sleep apnoea, polycystic ovary syndrome, sarcopenia, and frailty, particularly in older adults, reinforcing the concept of NAFLD/MASLD as a marker of systemic metabolic vulnerability [111].

5.1.6 *Clinical outcomes and disease progression of liver disease*

The clinical course of NAFLD is markedly heterogeneous, spanning from long-term stability to progressive liver-related morbidity and mortality and only a minority of individuals develop clinically significant disease progression [112],[113],[121].

Across epidemiological and prospective studies, the severity of liver fibrosis consistently emerges as the strongest determinant of adverse outcomes. All-cause mortality increases in a stepwise manner with advancing fibrosis stage, remaining relatively low in individuals with absent or mild fibrosis (F0–F2) and rising substantially in those with bridging fibrosis (F3) and cirrhosis (F4). Notably, mortality rates nearly double between stages F3 and F4, highlighting advanced fibrosis as a critical prognostic

threshold. Importantly, patients with bridging fibrosis already display a markedly increased incidence of hepatic decompensation and hepatocellular carcinoma (HCC) compared with those at earlier stages, suggesting that even modest fibrosis regression (for example, from F3 to F2) could yield meaningful reductions in liver-related events. The transition from simple steatosis to steatohepatitis represents a pivotal pathogenic step that accelerates fibrogenesis, and robust evidence indicates that fibrosis progression occurs predominantly in individuals with non-alcoholic steatohepatitis (NASH) [121].

5.1.7 *Therapeutic strategies and management principles of NAFLD*

Management of NAFLD/MASLD requires an integrated approach targeting both hepatic disease and systemic metabolic risk. Lifestyle modification remains the cornerstone of therapy, with sustained weight loss demonstrating dose-dependent improvements in steatosis, inflammation, and fibrosis. Physical activity independently reduces liver fat and improves cardiometabolic health, while alcohol reduction is strongly recommended due to synergistic liver toxicity [111]–[113].

Pharmacological options remain limited, but several agents targeting metabolic pathways show promise. Vitamin E and pioglitazone have demonstrated histological benefits in selected populations, while newer antidiabetic agents, particularly GLP-1 receptor agonists and SGLT2 inhibitors, are being actively investigated for their potential to modify both metabolic risk and liver disease progression. Importantly, statins are safe and essential for cardiovascular risk reduction, even in advanced liver disease [111]–[113].

5.1.8 *Lifestyle behaviour and NAFLD*

A substantial body of evidence supports a close and multifaceted association between lifestyle behaviours and the development and progression of non-alcoholic fatty liver disease [122],[123]. Sedentary behaviour and unhealthy dietary patterns represent key environmental drivers of metabolic dysfunction and NAFLD, acting independently and synergistically. Large epidemiological studies have demonstrated that prolonged sitting time is associated with a higher prevalence of NAFLD, even after adjustment for overall levels of physical activity, highlighting sedentary behaviour as an independent risk factor [124],[125]. Consistently, extended desk-working hours have been linked to an

increased incidence of NAFLD even among lean individuals, underscoring the pathogenic role of physical inactivity per se. When sedentary habits coexist with hypercaloric, nutrient-poor diets, they create a metabolic milieu characterised by positive energy balance and insulin resistance, which is central to hepatic lipid accumulation and disease progression [125].

Conversely, lifestyle interventions based on the combined implementation of regular physical exercise and caloric restriction, the so-called "dual method", have consistently demonstrated efficacy in preventing NAFLD and mitigating its progression [126]. This approach improves systemic insulin sensitivity, enhances energy expenditure, and reduces hepatic lipid overload, thereby addressing key mechanisms underlying disease pathogenesis [111],[125]. Beyond weight loss alone, healthy lifestyle patterns contribute to favourable changes in body composition, characterised by reductions in fat mass and preservation or increase of fat-free mass, which appear to be particularly relevant in protecting against NAFLD onset. Importantly, lifestyle modification has also been shown to influence the development and progression of liver fibrosis, currently the strongest prognostic determinant in NAFLD, reinforcing its central role in disease management [127].

In the absence of approved disease-specific pharmacological therapies, lifestyle modification remains the cornerstone of NAFLD treatment and a fundamental reference framework for personalised therapeutic strategies, as consistently recommended by clinical practice guidelines [111].

Nevertheless, despite robust evidence supporting the effectiveness of lifestyle-based interventions, their large-scale implementation in real-world settings remains limited. This gap between scientific evidence and clinical practice has become even more apparent during recent societal disruptions, such as the COVID-19 pandemic, when abrupt lifestyle changes characterised by reduced physical activity and unhealthy dietary habits further exacerbated metabolic risk profiles. Collectively, these observations emphasise that while effective non-pharmacological strategies for NAFLD are well established, addressing the societal and behavioural determinants of lifestyle change remains a critical and unresolved public health challenge.

5.2 Aims

This study aimed to elucidate the impact of short-term modifications in diet and physical activity associated with confinement measures on hepatic outcomes.

The primary endpoint was to evaluate the impact of SARS-CoV-2–related lifestyle modifications on changes in body composition and the worsening of metabolic syndrome components.

Secondary endpoints included the assessment of the pandemic's impact on hepatocellular carcinoma incidence and the identification of pandemic-related risk factors associated with HCC development.

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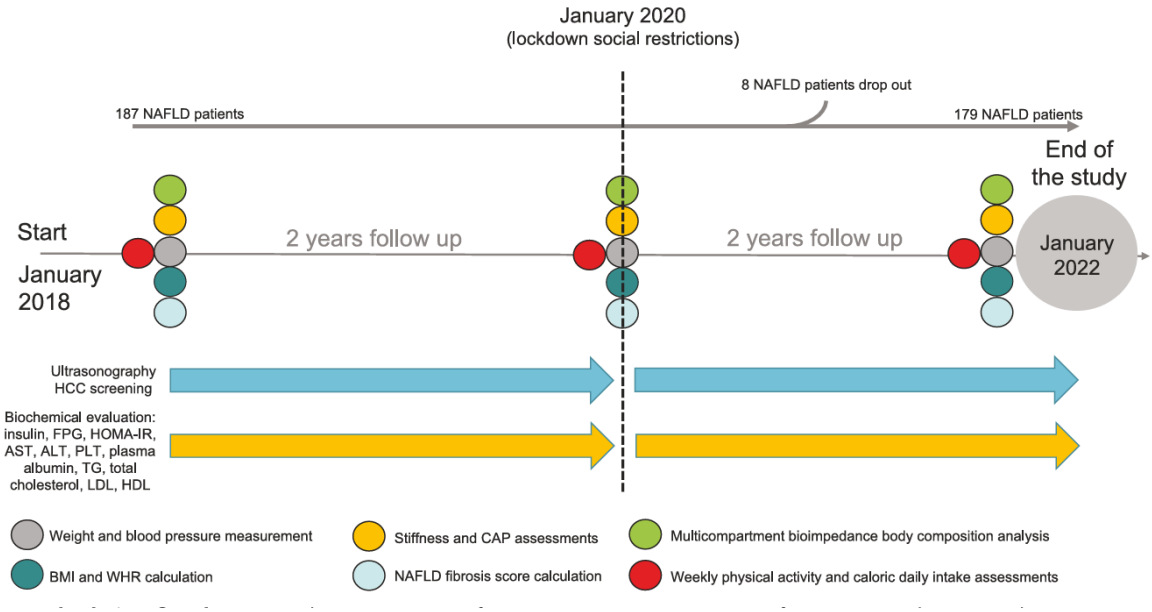
5.3 Methods

5.3.1 *Experimental design*

We conducted a four-year retrospective analysis of a cohort of patients with NAFLD, spanning from January 2018 to January 2022. The observation period was stratified according to the onset of lockdown-related social restrictions in January 2020 into three predefined time points: baseline (T0), the end of the pre-pandemic phase (January 2020, T1), and the end of follow-up (January 2022, T2). Throughout the study, patients underwent regular clinical, biochemical, and imaging evaluations in accordance with current clinical practice guidelines, with data expressed as mean values derived from assessments performed within each observation interval.

Hepatocellular carcinoma occurrence was systematically screened and recorded over the entire study period using ultrasonography, following guideline-based surveillance recommendations. The study design is illustrated in Figure 22. The study protocol was registered in the NIH U.S. National Library of Medicine clinical trials database (ClinicalTrials.gov identifier: NCT05416970).

Figure 22. Study design flowchart.



ALT Alanine Aminotransferase, AST Aspartate aminotransferase, BMI Body Mass Index, CAP Controlled Attenuation Parameter, FPG Fasting Plasma Glucose, HCC Hepatocellular carcinoma, HDL High-density lipoprotein, HOMA-IR Homeostatic model assessment for insulin resistance, LDL Low-density lipoprotein, NAFLD Non-alcoholic fatty liver disease, PLT Platelet count, TG triglycerides, WHR Waist-hip ratio.

5.3.2 *Study population*

This retrospective, multicentre, longitudinal study was conducted in accordance with the ethical principles of the Declaration of Helsinki (1975) and was approved by the Ethics Committee of the University of Campania "L. Vanvitelli", Naples (protocol no. 15.04-20220010000, approved on 29 March 2022). Between January 2018 and January 2022, adult patients with a diagnosis of non-alcoholic fatty liver disease, established on clinical, biochemical, imaging, and when available histological criteria in line with current clinical practice guidelines, were consecutively enrolled. Patients were followed at three tertiary referral centres: the Hepato-Gastroenterology Division of the University of Campania "Luigi Vanvitelli", the Internal Medicine and Hepatology Unit of the University of Salerno, and the Institute of Internal Medicine of the University of Foggia. All participants provided written informed consent prior to enrolment.

Inclusion criteria were age between 18 and 80 years and a confirmed diagnosis of NAFLD. Exclusion criteria included the presence of chronic inflammatory conditions (such as inflammatory bowel

disease, rheumatoid arthritis, or systemic lupus erythematosus), acute or chronic kidney disease, other major systemic diseases or active malignancies, ongoing infections, a history of alcohol or drug abuse, alternative causes of chronic liver disease, previous diagnosis of HCC, use of hepatoprotective drugs, insulin therapy (patients with type 2 diabetes mellitus were all insulin-naïve), and psychological or psychiatric conditions that could compromise the validity of informed consent.

A comprehensive medical history was collected for all participants. Alcohol consumption was longitudinally assessed using the full Alcohol Use Disorders Identification Test (AUDIT-C). Current medications particularly treatments for metabolic comorbidities including type 2 diabetes, obesity, arterial hypertension, and dyslipidaemia, were systematically classified and recorded over time, and drug abuse was specifically investigated.

Blood pressure was measured by trained physicians according to American Heart Association (AHA) recommendations [128]. Systolic and diastolic blood pressure were obtained using an aneroid sphygmomanometer with an appropriately sized cuff and a stethoscope, following the auscultatory Korotkoff method. For each participant, at least two measurements were obtained at intervals of no less than one minute, and the mean value was used for analysis; additional readings were performed when differences exceeded 5 mmHg, with the final value calculated as the average of multiple measurements.

Anthropometric assessment included measurement of body weight and height for calculation of body mass index (BMI) according to Quetelet's formula (kg/m^2) [129]. Waist and hip circumferences were measured using a flexible tape measure, and the waist-to-hip ratio (WHR) was calculated. Waist circumference was measured at the narrowest point between the rib cage and the iliac crest, while hip circumference was recorded at the widest point over the buttocks [129]. All anthropometric measurements were obtained in duplicate with participants standing and wearing light clothing, and values were rounded to the nearest 0.5 cm [129].

Biochemical parameters were collected throughout the study period and included fasting plasma glucose, insulin, the homeostasis model assessment of insulin resistance (HOMA-IR), aspartate aminotransferase, alanine aminotransferase, platelet count, plasma albumin, total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Insulin

levels were measured using commercially available enzymatic assays, while liver enzymes, lipid profile, and fasting glucose were assessed using standardized colorimetric methods. Plasma albumin concentration was determined using a bicinchoninic acid (BCA) protein assay. Platelet counts were measured with an automated haematology analyser based on electrical impedance technology. HOMA-IR was calculated using the standard formula: fasting insulin (μU/mL) × fasting plasma glucose (mmol/L) / 22.5 [130].

5.3.3 *Food intake, alcohol assumption, and physical exercise assessments*

At the end of the pre-pandemic period, as well as at the end of the study, dietary intake over a complete week including both working days and weekend days, was recorded for each participant and analysed using the WinFood software (Medimatica s.r.l., Martinsicuro, Italy). Based on both the quantity and quality of foods consumed, the software estimated the relative contributions of macronutrients and micronutrients for each food item and calculated total daily energy intake expressed as kilocalories per day, with specific attribution to carbohydrates, fats, and proteins. The comprehensive dietary analysis provided a detailed list of dietary components, the relative proportions among components, total caloric intake, and the distribution of energy intake across meals (breakfast, lunch, and dinner). The availability of an individualised list of dietary components for each patient enabled an expert dietician to further characterise dietary composition in terms of fat subtypes (saturated, monounsaturated, and polyunsaturated fats) and carbohydrate classes (monosaccharides, disaccharides, and polysaccharides).

Alcohol consumption was assessed using a standardised, pre-coded questionnaire (AUDIT-C test). Daily alcohol intake was quantified based on the concept of a “drink,” corresponding to approximately 12 g of pure ethanol [131]. Physical activity levels were evaluated using a structured, physician-assisted questionnaire.

5.3.4 *Non-invasive liver disease status evaluation: liver stiffness, controlled attenuation parameter, NAFLD fibrosis score*

Liver stiffness measurement (LSM) was obtained using transient elastography (TE) performed with the FibroScan® device, version 502 (Echosens, Paris, France), equipped with both M and XL probes [132]. The XL probe was used when the ultrasonographically measured distance between the skin and liver capsule exceeded 2.5 cm and/or when body mass index (BMI) was greater than 30 kg/m². All FibroScan® examinations were conducted by an experienced physician. A minimum of 10 valid measurements (defined as successful LSM acquisitions) were required, with a maximum of 20 attempts allowed. Measurement reliability was classified according to the criteria proposed by Boursier et al. as "very reliable" (interquartile range/median [IQR/M] ≤ 0.1), "reliable" (0.1 < IQR/M ≤ 0.3 or IQR/M > 0.3 with a median liver stiffness < 7.1 kilopascals [kPa]), or "poorly reliable" (IQR/M > 0.3 with a median liver stiffness ≥ 7.1 kPa) [132].

The controlled attenuation parameter (CAP), which quantifies ultrasonic attenuation in the liver at a frequency of 3.5 MHz, was assessed using signals acquired by the FibroScan® M and XL probes according to previously described physical principles [132]. CAP measurements were recorded only when obtained from valid acquisitions, using the same quality criteria applied for LSM [132],[133].

The NAFLD fibrosis score (NFS) was calculated using the following formula: −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m²) + 1.13 × type 2 diabetes mellitus (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (×10⁹/L) − 0.66 × plasma albumin (g/dL) [119].

5.3.5 *Multicompartment bioimpedance body composition analysis*

Body composition was assessed using a multifrequency bioelectrical impedance analysis (BIA) system (InBody, Seoul, Korea). For each measurement, two electrodes were placed on the right hand and two on the right foot. Participants were examined in the supine position after resting for at least 15 minutes. Measurements were performed in duplicate, and the mean of the two values was used for subsequent analyses. A low-intensity alternating electrical current at 50 kHz was passed through the body from hand to foot electrodes. Using measures of resistance (R), reactance (Xc), and phase angle

[arctangent (Xc/R) × (180/π)], the BIA system, through proprietary algorithms, estimated total body water (TBW), intracellular and extracellular water (ICW and ECW), fat-free mass (FFM), fat mass (FM), and body cell mass (BCM), expressed both as absolute values (kg) and percentages. The skeletal muscle mass index (SMMI) was calculated by dividing skeletal muscle mass (SMM) by height squared (m^2).

5.3.6 *Recording of HCC occurrence and definition of HCC overall and Milan-out criteria*

Hepatocellular carcinoma (HCC) surveillance was systematically performed using abdominal ultrasonography. HCC diagnosis was established in accordance with European Association for the Study of the Liver (EASL) clinical practice guidelines, based on contrast-enhanced ultrasound (CEUS) demonstrating typical imaging hallmarks of HCC (LI-RADS 5)[134]. At the time of diagnosis, HCC staging was defined using the Milan criteria [135]. Patients presenting with a single lesion measuring ≥2 cm and ≤5 cm, or up to three lesions each measuring ≥1 cm and ≤3 cm, in the absence of vascular invasion or extrahepatic metastases, were classified as "HCC Milan-in criteria." Conversely, patients not fulfilling these criteria were classified as "HCC Milan-out criteria." The term "HCC overall" referred to the combined occurrence of both "HCC Milan-in" and "HCC Milan-out" cases.

5.3.7 *Statistical analysis*

Baseline demographic characteristics were summarised using descriptive statistics. Continuous variables were expressed as means and standard deviations, while categorical variables were reported as counts and percentages. The Kolmogorov–Smirnov test was used to assess data normality and to determine whether parametric or non-parametric statistical tests were appropriate. Comparisons of continuous variables between two time points were performed using paired t-tests or Wilcoxon signed-rank tests, as appropriate. Comparisons across three time points were conducted using analysis of variance (ANOVA) with post hoc Tukey testing for normally distributed data, or the Kruskal–Wallis test for non-normally distributed data.

Time-to-event analyses for HCC occurrence and Milan-out staging at diagnosis were conducted using Kaplan–Meier survival curves and compared with the log-rank test, considering a p-value < 0.05 as statistically significant. The proportional hazards assumption was verified using Schoenfeld residuals. Odds ratios (ORs) for the association between study variables and the outcomes of interest were estimated using logistic regression models adjusted for potential confounders, including age, sex, BMI, type 2 diabetes mellitus, SARS-CoV-2 infection, and liver stiffness measurement. Sensitivity analyses were performed by stratifying the overall cohort according to age, sex, BMI, type 2 diabetes status, SARS-CoV-2 infection, and liver stiffness measurement. Statistical significance was defined as a two-tailed p-value < 0.05 with a 95% confidence interval. All analyses were conducted using the Statistical Package for Social Sciences (SPSS®), version 18.0.

5.4 Results

A total of 187 patients with NAFLD were enrolled and followed for four years. Overall, 179/187 participants completed the entire study period; eight patients discontinued during the pandemic because of HCC-related deaths.

Baseline characteristics of the study population are summarized in Table 12 .At baseline, the cohort included 97 women (51.9%) and 90 men (48.1%), with a mean age of 46.1 years. Type 2 diabetes mellitus (T2DM) was present in 74 patients (39.6%), and 62 patients (33.1%) experienced SARS-CoV-2 infection during follow-up. Based on liver stiffness measurement (LSM), 104/187 patients (55.6%) had LSM <10 kPa NAFLD (corresponding to F0–2 by METAVIR), whereas 83/187 (44.4%) had LSM ≥10 kPa; among these, 53 (28.3%) met criteria for compensated advanced chronic liver disease (cACLD) according to LSM >15 kPa.

Table 12. Clinical characteristics of the study population.

Variables	n (%)
Sex (Female)	97 (51.9%)
Age	46.1 (12.79)
SARS-CoV-2 infection during the observation period	62 (33.1%)
Type2 Diabetes Mellitus (T2DM)	74 (39.6%)
T2D, on-treatment	71 (95.9%)

- Metformin	32 (45.1%)
- Sulfonylureas	20 (28.2%)
- Gliptins	19 (26.7%)
Dyslipidemia	83 (44.4%)
Dyslipidemia, on-treatment	74 (89.1%)
- Statins	43 (58.1%)
- Ezetimibe	22 (29.7%)
- Statin/Ezetimibe	9 (12.1%)
- Fibrates	15 (20.3%)
Obesity	98 (52.4%)
Obesity, on-treatment	16 (16.3%)
- DPP4-I	9 (56.2%)
- GLP-1 agonist	5 (31.3%)
- Intestinal lipase-I (orlistat)	2 (12.5%)
Hypertension	92 (49.2%)
Hypertension, on-treatment	75 (81.5%)
- Diuretics	37 (49.3%)
- ACE-I	22 (29.3%)
- CCB	11 (14.6%)
- Beta-Blockers	13 (17.3%)
- Sartans	19 (25.3%)

Data are reported as frequencies and distribution (%). Age is reported as mean±SD. ACE-I Angiotensin-converting enzyme inhibitors, CCB Calciumchannel blockers, DPP4-I dipeptidylpeptidase inhibitors, GLP-1glucagon-likepeptide1.

Comparison of anthropometric indices, biochemical parameters, and non-invasive measures of fibrosis/steatosis (NFS, LSM, and CAP) across three-time points evaluations is reported in Table 13. Regarding anthropometrics, BMI, WHR, and WHR/BMI increased significantly when comparing T0 and T1 with the end of the study ($p<0.0001$ for BMI and WHR; $p<0.001$ for WHR/BMI), whereas no significant variations were observed in SBP or DBP (Table 1). Among biochemical variables, total cholesterol, LDL, triglycerides, insulin, glucose, HOMA-IR, AST, and ALT increased significantly, while HDL decreased ($p<0.0001$ for all variables except glucose: $p=0.005$). Consistently, non-invasive markers of liver disease progression worsened over time: NFS, LSM, and CAP were all significantly higher at the end of follow-up compared with T0 and T1 ($p<0.0001$ for all) (Table 13, Fig. 23).

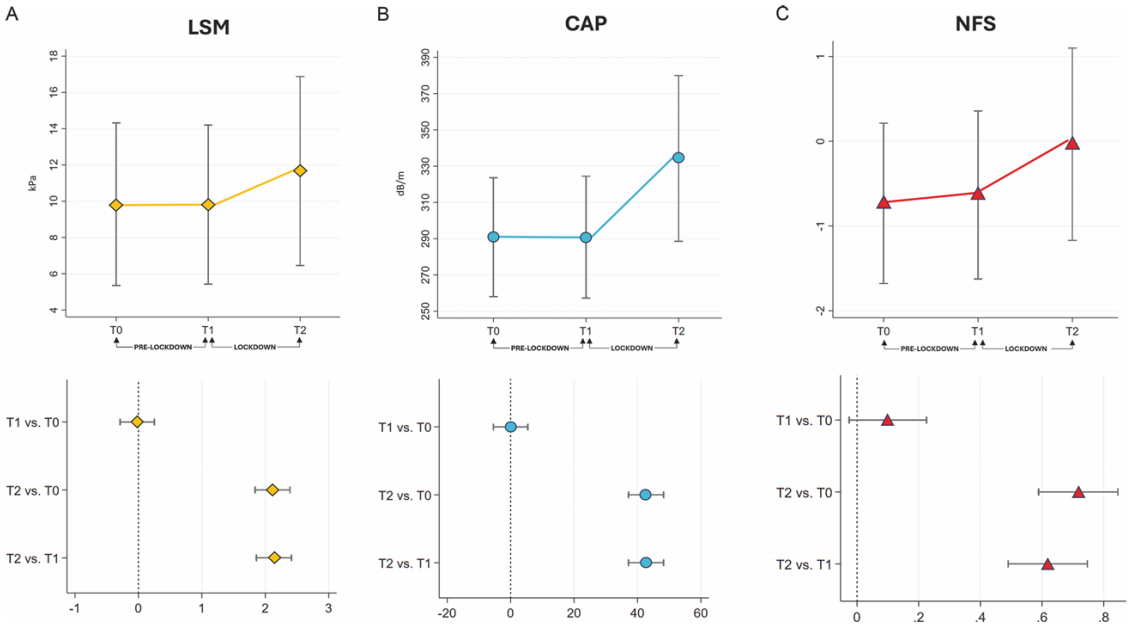
Table 13. Comparison of anthropometric indices, biochemical parameters, and non-invasive measures of fibrosis/steatosis across three-time points evaluations.

Variables	Baseline (T0: January 2018)	Intermediate (T1: January 2020)	End of the study (T2: January 2022)	Comparison between the three-time points evaluations		
				Time-points	*p*-value	95% CI
Height (m)	1.69 ± 0.07	/	/		/	
Weight (kg)	79.6 ± 9.6	79.4 ± 10.12	88.3 ± 14.9	T0 vs T1	0.82	-0.47 to 0.78
				T0 vs T2	**<0.0001**	-8.97 to -4.42
				T1 vs T2	**<0.0001**	-9.04 to -4.66
Body Mass Index	27.8 ± 2.2	27.7 ± 2.4	30.1 ± 4.6	T0 vs T1	0.8	-0.16 to 0.28
				T0 vs T2	**<0.0001**	-3.05 to -1.45
				T1 vs T2	**<0.0001**	-3.08 to -1.54
SBP (mmHg)	132.1 ± 12	131.2 ± 9.4	130.5 ± 9.4	T0 vs T1	0.43	-0.76 to 2.43
				T0 vs T2	0.07	-0.10 to 3.31
				T1 vs T2	0.44	-0.71 to 2.26
DBP (mmHg)	78.7 ± 10.9	78.4 ± 9.5	78.6 ± 8.4	T0 vs T1	0.91	-1.36 to 2.01
				T0 vs T2	0.98	-1.67 to 1.92
				T1 vs T2	0.95	-1.76 to 1.37
WHR	0.97 ± 0.13	0.97 ± 0.13	1.13 ± 0.22	T0 vs T1	0.99	-0.02 to 0.02
				T0 vs T2	**<0.0001**	-0.20 to -0.12
				T1 vs T2	**<0.0001**	-0.20 to -0.12
Total cholesterol (mg/dL)	185.2 ± 17.2	183.8 ± 16.3	223.2 ± 28.2	T0 vs T1	0.79	-1.40 to 2.46
				T0 vs T2	**<0.0001**	-44.60 to -32.59
				T1 vs T2	**<0.0001**	-44.94 to -33.31
LDL (mg/dL)	118.9 ± 21.2	118.3 ± 20.05	162.5 ± 30.3	T0 vs T1	0.929	-3.01 to 4.11
				T0 vs T2	**<0.0001**	-49.55 to -37.70
				T1 vs T2	**<0.0001**	-49.88 to -38.47
HDL (mg/dL)	43.7 ± 11.8	43.7 ± 10.4	30.4 ± 9.2	T0 vs T1	0.99	-1.89 to 1.85
				T0 vs T2	**<0.0001**	11.04 to 15.66
				T1 vs T2	**<0.0001**	11.21 to 15.53
Triglycerides (mg/dL)	110.8 ± 26.2	111.2 ± 26.8	153.4 ± 43.8	T0 vs T1	0.99	-4.41 to 4.34
				T0 vs T2	**<0.0001**	-47.76 to -33.70
				T1 vs T2	**<0.0001**	-47.47 to -33.95
Insulin (□U/mL)	22.01 ± 11.4	23.02 ± 11.8	24.4 ± 12.6	T0 vs T1	0.8143	-1.60 to 1.31
				T0 vs T2	**0.0003**	10.76 to 14.70
				T1 vs T2	**0.0003**	10.63 to 14.39
Glucose (mmol/L)	4.8 ± 1.6	4.9 ± 1.7	5.1 ± 1.8	T0 vs T1	0.8366	-1.40 to 1.42
				T0 vs T2	**0.0007**	10.26 to 12.80
				T1 vs T2	**0.0007**	10.42 to 12.19
HOMA-IR	5.13 ± 3.85	5.92 ± 3.91	6.15 ± 4.41	T0 vs T1	0.7892	-1.30 to 1.52
				T0 vs T2	**<0.0001**	11.76 to 12.70
				T1 vs T2	**<0.0001**	10.13 to 13.59

AST (U/L)	40.9 ± 15.1	40.1 ± 13.5	54.5 ± 15.6	T0 vs T1	0.699	-1.49 to 3.04
				T0 vs T2	**<0.0001**	-16.65 to -10.56
				T1 vs T2	**<0.0001**	-17.33 to -11.43
ALT (U/L)	70.5 ± 15.3	69.4 ± 15.7	87.4 ± 21.9	T0 vs T1	0.1354	-1.41 to 3.67
				T0 vs T2	**<0.0001**	-20.32 to -13.56
				T1 vs T2	**<0.0001**	-21.94 to -14.21
PLT ($x10^3$/□L)	157.7 ± 34.5	158.2 ± 34.4	153.1 ± 40.8	T0 vs T1	0.7320	-2.11 to 1.09
				T0 vs T2	0.0552	6.49 to 15.09
				T1 vs T2	0.1334	7.49 to 15.11
Albumin (g/dL)	3.6 ± 0.3	3.6 ± 0.3	3.5 ± 0.5	T0 vs T1	0.3064	-0.01 to 0.04
				T0 vs T2	0.1225	0.10 to 0.21
				T1 vs T2	0.0576	0.09 to 0.19
NFS	-0.73 ± 0.95	-0.63 ± 0.99	-0.03 ± 1.13	T0 vs T1	0.072	-0.21 to 0.01
				T0 vs T2	**<0.0001**	-0.83 to -0.57
				T1 vs T2	**<0.0001**	-0.74 to -0.45
LSM (kPa)	9.85 ± 4.5	9.82 ± 4.38	11.68 ± 5.21	T0 vs T1	0.847	-0.07 to 0.12
				T0 vs T2	**<0.0001**	-2.16 to -1.50
				T1 vs T2	**<0.0001**	-2.20 to -1.51
CAP (dB/m)	290.8 ± 32.8	291.0 ± 33.6	334.3 ± 45.7	T0 vs T1	>0.99	-1.79 to 1.80
				T0 vs T2	**<0.0001**	-50.16 to -36.70
				T1 vs T2	**<0.0001**	-50.07 to -36.78

ALT alanine aminotransferase, AST aspartate aminotransferase, DBP Diastolic Blood Pressure, CAP controlled attenuation parameter, dL deciliter, HDL high density lipoprotein, HOMA-IR homeostasis model assessment for insulin resistance, kPa Kilopascal, L liter, LSM Liver stiffness measurement, LDL Low-density lipoprotein,Mmeter,mmolmillimoles,mmHgmillimeters:millimeterofmercury,NFS NAFLD Fibrosis Score, PLT platelets, SBP Systolic Blood Pressure, SD standard deviation, U unit, WHR waist-hip ratio, µ micro, n number.

Figure 23. Hepatopathy progression non-invasive tools modifications.



*(A) LSM: Liver stiffness measurement, (B) CAP: Controlled attenuation parameter, (C) NFS: NAFLD Fibrosis score comparison among the three study time points by using ANOVA and Tukey post hoc analysis. kPa: Kilopascal; db/m: decibel/meter. *p < 0.0001.*

Changes in dietary and physical activity habits across study time points are detailed in Table XXX. Time spent in physical activity declined significantly at T2 compared with both T1 and T0 ($p<0.0001$ for both comparisons). Conversely, daily caloric intake increased significantly from T0 and T1 to T2 ($p<0.0001$ for both). In parallel with these quantitative changes, macronutrient intake (kcal/day) also shifted significantly: carbohydrate and lipid contributions increased, whereas protein intake decreased, when comparing T0 and T1 with the end of follow-up ($p<0.0001$ for all). In contrast, the proportional intake of carbohydrate subtypes (monosaccharides, disaccharides, polysaccharides) and fat subtypes (saturated, monounsaturated, polyunsaturated), as well as AUDIT-C–estimated daily alcohol intake, did not change significantly over the study period.

Table 14. Assessment of physical activity, nutritional and dietary habits among the three-time points evaluations.

Variables	Baseline (T0: January 2018)	Intermediate (T1: January 2020)	End of the study (T2: January 2022)	Comparison between the three-time points evaluations		
				Time-points	p-value	95% CI
Physical activity (hours/week)	6.1 ± 1.3	6.1 ± 1.2	4.3 ± 1.4	T0 vs T1	0.993	-0.20 to 0.22
				T0 vs T2	**<0.0001**	1.53 to 2.20
				T1 vs T2	**<0.0001**	1.52 to 2.19
Alcohol intake* ("Drink"/day)	1.4 ± 0.3	1.7 ± 0.2	1.5 ± 0.4	T0 vs T1	0.989	-51.90 to 61.39
				T0 vs T2	0.994	-54.20 to 61.47
				T1 vs T2	0.991	-56.30 to 64.81
Daily intake (Kilocalories/ day)	2296 ± 397.1	2292 ± 401.9	3024 ± 545.4	T0 vs T1	0.986	-59.20 to 67.49
				T0 vs T2	**<0.0001**	-847.30 to -607.60
				T1 vs T2	**<0.0001**	-845.40 to -617.80
Carbohydrates (Kilocalories)	1069 ± 227	1069 ± 235	1716 ± 414.1	T0 vs T1	> 0.99	-36.49 to 36.64
				T0 vs T2	**<0.0001**	-727.50 to -566.10
				T1 vs T2	**<0.0001**	-727.50 to -566.20
Lipids (Kilocalories)	566.6 ± 126.9	574.4 ± 126.7	1055 ± 261.4	T0 vs T1	0.655	-28.72 to 13.17
				T0 vs T2	**<0.0001**	-538.50 to -438.10
				T1 vs T2	**<0.0001**	-528.90 to -432.20
Proteins (Kilocalories)	661 ± 185.1	649.2 ± 198.9	234.3 ±166.9	T0 vs T1	0.62	-18.18 to 41.86
				T0 vs T2	**<0.0001**	387.00 to 466.50
				T1 vs T2	**<0.0001**	375.40 to 454.50

**Assessed by using AUDIT-C test (one drink corresponds to about 12 g of pure ethanol); SD: standard deviation. The Kruskal-Wallis test or ANOVA test with post-hoc Tukey analysis, in the case of non-normal or normal distribution respectively, were performed to compare the continuous variables among three observation times. Statistically significant differences ($p<0.05$) among the three periods are reported in bold.*

Macronutrient distribution changed across evaluation time points, with an increase in carbohydrates (T0: 46.5% vs. T1: 46.6% vs. T2: 57.4%) and fats (T0: 24.7% vs. T1: 25.1% vs. T2: 34.7%), alongside a reduction in proteins (T0: 28.8% vs. T1: 28.3% vs. T2: 7.9%). No increase in alcohol consumption was observed over the study period.

Body composition analysis showed a significant increase in fat mass (FM), both in kilograms and as a percentage of total body compartments, together with a reduction in fat-free mass (FFM) percentage

at the end of the study compared with earlier time points (p<0.0001 for all). Similarly, extracellular mass (ECM) and body cell mass (BCM) decreased significantly, both in kilograms and percentage values, when comparing the end of follow-up with previous assessments (p<0.001 for all) (Table 15).

Table 15. Assessment of body composition parameters among the three-time points evaluations.

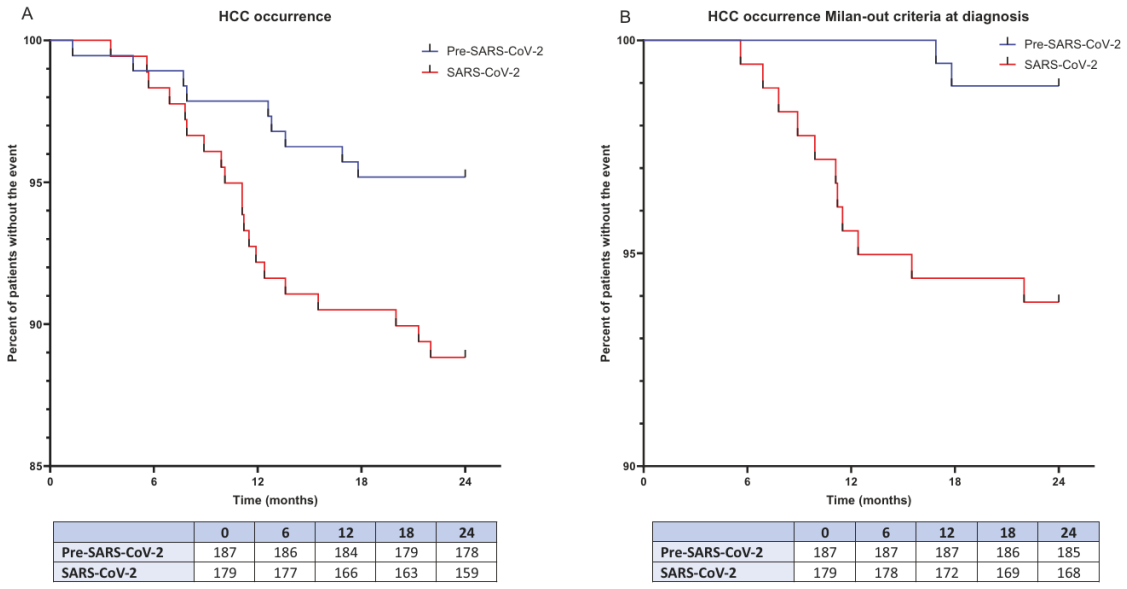
Variables	Baseline (T0: January 2018)	Intermediate (T1: January 2020)	End of the study (T2: January 2022)	Comparison between the three-time points evaluations		
				Time-points	p-value	95% CI
FFM (Kg)	63.4 + 7.9	63.3 + 8.2	63.7 + 11.1	T0 vs T1	0.847	-0.39 to 0.62
				T0 vs T2	0.862	-1.97 to 1.26
				T1 vs T2	0.767	-2.08 to 1.14
FFM (%)	79.7 + 4	79.7 + 3.9	74 + 6.3	T0 vs T1	0.991	-0.35 to 0.31
				T0 vs T2	**<0.0001**	4.63 to 6.59
				T1 vs T2	**<0.0001**	4.68 to 6.57
SMMI	10.4 + 1.1	10.3 + 1.1	10.4 + 1.6	T0 vs T1	0.979	-0.07 to 0.08
				T0 vs T2	0.877	-0.29 to 0.19
				T1 vs T2	0.846	-0.29 to 0.18
FM (Kg)	16.2 + 3.9	16.2 + 3.9	22.5 + 7.6	T0 vs T1	0.962	-0.29 to 0.36
				T0 vs T2	**<0.0001**	-7.53 to -5.11
				T1 vs T2	**<0.0001**	-7.51 to -5.21
FM (%)	20.3 + 4.01	20.3 + 3.9	26 + 6.3	T0 vs T1	0.992	-0.31 to 0.35
				T0 vs T2	**<0.0001**	-6.59 to -4.64
				T1 vs T2	**<0.0001**	-6.56 to -4.68
ECM (Kg)	31.3 + 5.8	31.3 + 5.8	31.1 + 7.2	T0 vs T1	0.882	-0.31 to 0.46
				T0 vs T2	**0.0003**	-2.82 to -0.72
				T1 vs T2	**0.0001**	-2.88 to -0.82
ECM (%)	39.26 + 4.8	39.3 + 4.7	35.9 + 6.2	T0 vs T1	0.994	-0.35 to 0.38
				T0 vs T2	**<0.0001**	-3.15 to -1.88
				T1 vs T2	**<0.0001**	-3.04 to -2.03
BCM (Kg)	25.5 + 3.4	25.5 + 3.6	24.1 + 4.9	T0 vs T1	0.944	-0.25 to 0.33
				T0 vs T2	**<0.0001**	0.64 to 2.18
				T1 vs T2	**<0.0001**	0.66 to 2.09
BCM (%)	40.4 + 3.5	40.3 + 3.5	37.8 + 4	T0 vs T1	0.994	-0.38 to 0.35
				T0 vs T2	**<0.0001**	1.88 to 3.15
				T1 vs T2	**<0.0001**	2.03 to 3.04
TBW (%)	46.3 + 5.9	46.1 + 5.8	46.7 + 7.3	T0 vs T1	0.855	-0.67 to 1.06
				T0 vs T2	0.79	-1.74 to 0.99

	T1 vs T2	0.599	-1.97 to 0.83

BCM, body cell mass; ECM, extracellular mass; FM, fat mass; FFM, free fat mas.; SMM, skeletal muscle mass; SMMI, skeletal muscle mass index; SD, standard deviation; TBW, total body water. Statistically significant differences between the three periods are reported in bold. The Kruskal-Wallis test or ANOVA test with post-hoc Tukey analysis, in the case of non-normal or normal distribution respectively, were performed to compare the continuous variables among three times of observation. Statistically significant differences ($p<0.05$) among the three periods are reported in bold.

HCC occurrence increased significantly during the lockdown period. Between January 2018 and January 2020, HCC occurred in 9/187 patients (incidence rate 4.8%); among these, 2 cases were staged beyond Milan criteria at diagnosis (Milan-out rate among HCC cases: 22.2%). In the subsequent period (January 2020 to January 2022), 20/179 patients developed HCC (incidence rate 11.1%), and 11 of these presented with Milan-out stage at diagnosis (rate among HCC cases: 55%). Owing to lockdown restrictions, 39/179 participants postponed abdominal ultrasound surveillance for HCC screening, with delays ranging from 30 to 112 days; however, none of these patients developed HCC during the study period. Kaplan–Meier analyses using the log-rank test indicated a higher risk during lockdown for both overall HCC occurrence (HR: 2.39, 95% CI: 1.16–5, p=0.02) and Milan-out stage at diagnosis (HR: 5.9, 95% CI: 2–17.6, p=0.008) (Fig. 24 A,B).

Figure 24. Hepatocellular carcinoma occurrence in the period preceding the lockdown and during the lockdown.



	0	6	12	18	24
Pre-SARS-CoV-2	187	186	184	179	178
SARS-CoV-2	179	177	166	163	159

	0	6	12	18	24
Pre-SARS-CoV-2	187	187	187	186	185
SARS-CoV-2	179	178	172	169	168

The Kaplan-Meier Log-Rank Test analysis comparing the HCC overall (A) and Milan-out criteria at diagnosis (B) occurrence pre- vs during the lockdown.

Logistic regression analyses for both overall HCC occurrence and Milan-out stage at diagnosis during the lockdown period was performed. Among biochemical variables, only ALT was significantly associated with overall HCC occurrence (p=0.045, OR: 1.03, 95% CI: 1.001–1.061), whereas insulin (p=0.766), glucose (p=0.85), HOMA-IR (p=0.918), AST (p=0.77), total cholesterol (p=0.6), TG (p=0.474), HDL (p=0.665), and LDL (p=0.626) were not. In contrast, multiple body composition indices showed significant associations with overall HCC occurrence: FFM (kg and %), BCM (kg and %), and SMMI were inversely associated (p<0.0001 for all, except SMMI: p=0.004), while FM (kg and %) was positively associated (p<0.0001 for both).

For Milan-out stage at diagnosis during lockdown-related lifestyle changes, biochemical variables again showed no significant associations (insulin: p=0.08; glucose: p=0.152; HOMA-IR: p=0.091; AST: p=0.071; ALT: p=0.198; total cholesterol: p=0.673; TG: p=0.537; HDL: p=0.669; LDL: p=0.672). Conversely, FM (kg and %) was positively associated (p=0.002 for both), while FFM (kg and %), BCM (kg and %), and SMMI were inversely associated (p=0.01, p=0.002, p=0.01, p=0.003, and p=0.04, respectively). LSM was also inversely associated with this outcome (p=0.03). During the pre-lockdown period, none of the assessed biochemical parameters, body composition measures, or non-invasive stiffness tools were associated with Milan-out stage at diagnosis (Table 16).

Table 16. Multinomial logistic regression analysis showing the variables significantly associated with HCC overall and HCC staged Milan-out criteria at diagnosis occurrence during the lockdown.

Variables	Outcome: HCC overall occurrence during the lockdown			Outcome: HCC staged Milan-out criteria at diagnosis during the lockdown		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
FFM (Kg)	0.81	0.72-0.91	**0.0003**	0.81	0.69-0.95	**0.01**
FFM (%)	0.70	0.60-0.83	**<0.0001**	0.69	0.54-0.87	**0.002**
SMMI	0.57	0.39-0.83	**0.004**	0.560	0.36-0.98	**0.04**
BCM (Kg)	0.54	0.40-0.72	**<0.0001**	0.58	0.41-0.83	**0.003**
BCM (%)	0.66	0.53-0.83	**0.0003**	0.72	0.55-0.94	**0.01**
FM (Kg)	1.33	1.16-1.53	**<0.0001**	1.36	1.12-1.66	**0.002**
FM (%)	1.42	1.21-1.67	**<0.0001**	1.46	1.15-1.84	**0.002**
LSM (kPa)	0.85	0.75-0.96	**0.01**	0.82	0.69-0.98	**0.03**

Confounding variables (age, sex, BMI, T2DM, SARS-CoV-2 infection, and LSM.
BCM: Body cellular mass; FFM: Free fat mass; FM: Fat mass; LSM: Liver stiffness measurement; kPa: Kilopascal; Kg: kilograms; SMM: Skeletal muscle mass; SMMI: Skeletal muscle mass index.

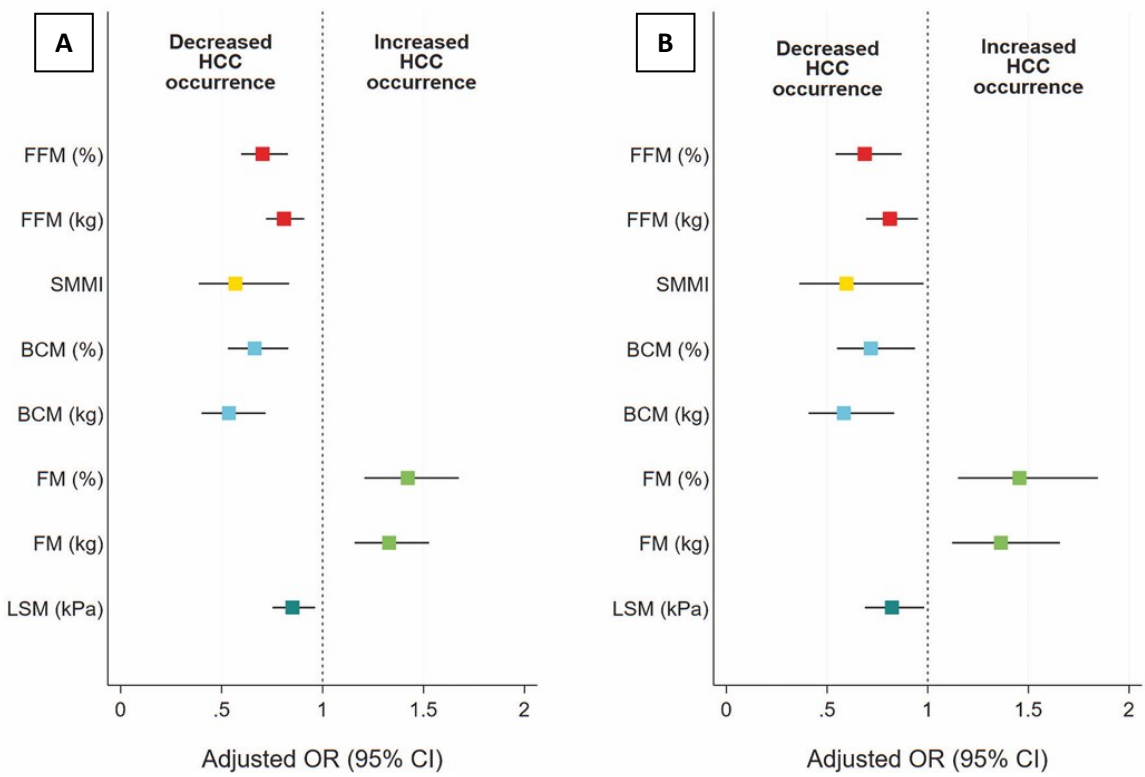
Finally, we evaluated whether the magnitude of change between T1 and T2 (Δ) in the significant parameters was associated with lockdown-period outcomes (Fig. 25 and Table 17). ΔFM (kg and %) was positively associated with both overall HCC occurrence and Milan-out stage at diagnosis, whereas ΔFFM (kg and %), ΔBCM (kg and %), ΔSMMI, and ΔLSM were inversely associated with both outcomes. By contrast, delta values calculated between T0 and T1 (Δ2) did not show significant associations with HCC occurrence during the pre-lockdown period (Δ2 FM kg: p=0.426; Δ2 FM %: p=0.248; Δ2 FFM kg: p=0.186; Δ2 FFM %: p=0.248; Δ2 BCM kg: p=0.062; Δ2 BCM %: p=0.195; Δ2 SMMI: p=0.054; Δ2 LSM: p=0.302).

Table 17. Multinomial logistic regression analysis of the delta values (January 2020 vs end of the study assessments) of the parameters significantly associated with HCC overall and HCC staged Milan-out criteria at diagnosis occurrence during the lockdown.

Variables	Outcome: HCC overall occurrence during the lockdown			Outcome: HCC staged Milan-out criteria at diagnosis during the lockdown		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Δ FFM (Kg)	0.58	0.44-0.75	**<0.0001**	0.61	0.46-0.82	**0.001**
Δ FFM (%)	0.78	0.69-0.88	**<0.0001**	0.80	0.58-0.86	**0.001**
Δ SMMI	0.57	0.35-0.93	**0.02**	0.46	0.22-0.92	**0.02**
Δ BCM (Kg)	0.46	0.33-0.64	**<0.0001**	0.21	0.07-0.64	**0.007**
Δ BCM (%)	0.56	0.44-0.73	**<0.0001**	0.48	0.33-0.71	**0.0002**
Δ FM (Kg)	1.51	1.24-1.85	**<0.0001**	1.42	1.13-1.78	**0.003**
Δ FM (%)	1.74	1.33-2.26	**<0.0001**	1.63	1.22-2.18	**0.001**
Δ LSM (kPa)	0.40	0.25-0.64	**0.0001**	0.30	0.14-0.63	**0.002**

Confounding variables (age, sex, BMI, T2DM, SARS-CoV-2 infection and LSM.
Δ= variation January 2020 vs end of the study assessments; BCM: Body cellular mass; FFM: Free fat mass; FM: Fat mass; LSM: Liver stiffness measurement; kPa: Kilopascal; Kg: kilograms; SMM: Skeletal muscle mass; SMMI: Skeletal muscle mass index.

Figure 25. Body composition variables and relative association with HCC occurrence during the lockdown (A) and HCC staged Milan-out criteria at diagnosis during lockdown (B).



Adjusted Odds Ratios for bioimpedance analysis factors' variations on hepatocellular carcinoma (HCC) overall (A) and Milan-out criteria at diagnosis (B) occurrence during the lockdown period.

Sensitivity analyses stratified by age, sex, obesity status, diabetes status, and SARS-CoV-2 status yielded consistent results with the main analysis and are not reported here.

5.5 Discussion

The growing global burden of metabolic-associated liver diseases and their comorbidities represents a critical priority for the scientific community. In the absence of approved effective therapies, despite the risk of rapid progression to HCC, NAFLD remains a major unmet clinical challenge. Over the last two years, populations worldwide experienced profound lifestyle changes driven, from a medical standpoint, by SARS-CoV-2 diffusion and the consequent government-imposed measures aimed at limiting contagion, with broad societal repercussions [136].

Within this exceptional context, the European spread of SARS-CoV-2 acted as a trigger for unhealthy dietary and physical activity behaviours, providing NAFLD with a clear disadvantage. Our NAFLD

cohort was prospectively monitored for two years before and two years after the introduction of lockdown-related social restrictions, documenting marked lifestyle changes, including increases in total daily caloric intake and modifications in dietary composition, alongside reduced regularity and weekly time devoted to physical exercise. Quarantine measures have been associated with short-term weight gain, particularly among individuals with pre-existing risk factors such as more frequent snacking, reduced water intake, emotional eating, poorer sleep, and overweight/obesity [137]. Nevertheless, the extent to which this phenomenon accelerates NAFLD progression remains insufficiently defined, as it should be incorporated into a more complex risk model that also accounts for body composition changes and the time frame over which these modifications occur.

Overall, our results showed a deterioration in several metabolic parameters at the end of lockdown compared with the preceding periods (T2 vs T1 & T0). Specifically, we observed a worsening of disease stage as assessed by both LSM and NFS, together with increased CAP. The concomitant deterioration of multiple biochemical parameters further supported an overall derangement of the clinical profile during lockdown, outlining a complex social-health scenario in which liver disease progression is promoted. To our knowledge, this study represents the first observation addressing the impact of lockdown-imposed lifestyle changes on NAFLD clinical progression and outcomes.

In a retrospective longitudinal study including 973 individuals undergoing health check-ups between 2018 and 2020, Fujii et al. identified independent predictors of metabolic dysfunction-associated fatty liver disease (MAFLD) development during the pandemic observation window (2019–2020), with daily alcohol intake and, specifically among subjects aged <60 years, the proportion of participants eating twice per day emerging as statistically significant factors [138]. However, that study did not report data on potential worsening among patients already diagnosed at baseline; moreover, in our cohort, alcohol consumption did not significantly change over the entire study period.

A bidirectional relationship between SARS-CoV-2 infection and NAFLD has been reported, potentially driven by overlapping inflammatory pathways, while underlying liver fibrosis may independently increase the risk of severe COVID-19. In our cohort, despite several infections during the pandemic, SARS-CoV-2 was not significantly associated with deterioration of the assessed parameters or with adverse outcomes such as HCC development or death. To date, sequential data on

body composition trajectories in NAFLD patients throughout the pandemic have not been previously reported. Here, we documented substantial shifts in body compartments in the study population, with a marked increase in FM (percentage and kg) together with reductions in FFM (percentage) and BCM (percentage). An observational study assessed pandemic-related changes in body composition among children and adolescents, showing overall stability in muscle-to-fat ratio, with improvements mainly in underweight and normal-weight individuals but not in those with overweight/obesity. Socioeconomic status, pre-pandemic anthropometric and body-composition measures, and physical activity during the pandemic emerged as key predictors of these changes [139]. Overall, these data suggest that individuals with overweight/obesity may be more vulnerable to unfavourable body-composition shifts.

With respect to dietary composition, carbohydrate and lipid proportions increased significantly from the T0 and T1 evaluations to the end of the study, whereas protein contribution decreased significantly. Previous studies have explored the metabolically harmful effects of simple carbohydrates (monosaccharides and disaccharides) on insulin resistance and hepatic steatosis and have suggested that excessive saturated fat intake contributes to hepatic and visceral fat accumulation, while polyunsaturated fat intake may promote lean tissue in humans.

Regarding physical exercise, consistently with prior evidence [139], time spent in physical activity was significantly reduced at T2 compared with both T1 and T0. Notably, multiple studies have also identified sedentary behavior as an independent determinant of NAFLD progression, indicating that prolonged sitting time may contribute per se to NAFLD worsening and associated cardiometabolic comorbidities, independently of reduced exercise levels [124]. Although validated questionnaires assessing both physical activity and sedentary habits are available, and to avoid an “horror vacui”–driven approach, sitting time was not included and was not directly assessed within our study design. Nevertheless, given the specific context of pandemic-related restrictions forcing participants into “home life”, and considering that home-based sitting activities (including, among others, social network use and internet gaming) plausibly accounted for most of the time during a “typical lock-down day”, sedentary exposure can be reasonably inferred as the complement of time spent in physical exercise and therefore should not be regarded as missing information in our research.

We also observed a marked increase in HCC occurrence and in the proportion of "Milan-out" stage at diagnosis during the lockdown compared with the earlier study period. This finding is particularly concerning considering that, prior to the pandemic, the global incidence of NAFLD-related HCC ranged from 0.5% to 2.6% among patients with NASH cirrhosis and was even lower in non-cirrhotic NAFLD (approximately 0.1 to 1.3 per 1000 patient-years) [140]. In this regard, reduced adherence to screening programs recommended by clinical practice guidelines (CPG), due to exposure concerns and resource reallocation, appeared not to substantially influence the findings [111]. Conversely, our logistic regression model confirmed LSM as an independent major risk factor for HCC occurrence in the pre-pandemic period and, importantly, revealed an unexpected contribution of body composition changes to both HCC occurrence and "Milan-out" stage at diagnosis during lockdown. Specifically, FM (kg and percentage), FFM (kg and percentage), SMMI, and BCM (kg and percentage) were significantly associated with both outcomes, independently of age, BMI, sex, T2DM, LSM, SARS-CoV-2 infection, CAP, and other covariates.

Furthermore, the odds ratio estimates gained additional relevance when considering delta changes (T2 vs T1) in these parameters, reinforcing the link between their deterioration and HCC risk. Unexpectedly, during the pandemic period LSM showed less strength in influencing the evaluated outcomes. This result should be interpreted cautiously, since most newly diagnosed HCC cases during the pandemic occurred in non-cACLD patients (n. 16/20), and larger prospective observational studies may provide further clarification.

In light of our findings, the extent to which the rapid body composition changes observed here may influence NAFLD natural history, complication onset, and HCC evolution, also in terms of treatment response, remains an open unmet question. The retrospective design represents a key limitation, and a larger multicenter observational study could help corroborate these results. A longer follow-up would also improve the evaluation of the true long-term impact of the pandemic in this setting.

Overall, in our study, acute lockdown-related lifestyle changes reduced physical activity and increased caloric intake, were associated with worsening anthropometric, biochemical, body-composition, and non-invasive liver disease measures, alongside higher HCC occurrence (including "Milan-out" stage

at diagnosis), with body-compartment alterations emerging as the main predictor and a potentially useful tool for NAFLD-tailored risk stratification.

6. CONCLUSIONS

6.1 Summary of main findings

This PhD thesis investigated the relationship between metabolic dysfunction, systemic inflammation, and chronic disease through three complementary studies that, while addressing distinct clinical contexts share a common conceptual framework centred on the extent to which metabolic and inflammatory factors shape disease expression, modify diagnostic precision, and determine clinical outcomes across conditions of growing public health relevance. Together, the three studies provide convergent evidence that metabolic status is not a passive comorbidity but an active biological determinant of disease behaviour, and that accounting for it, whether in therapeutic decision-making, diagnostic classification, or prognostic risk stratification, is essential for a more accurate and personalised approach to chronic disease management.

Study 1 demonstrated that obesity, despite being associated with a distinct clinical and inflammatory profile characterised by a relatively attenuated type-2 inflammatory signature, does not impair the long-term effectiveness of biologic therapy in severe asthma. Across nearly 2,100 patients from the SANI registry, obese and non-obese patients achieved sustained and comparable improvements in exacerbation frequency, asthma control, quality of life, lung function, and clinical remission over up to four years of follow-up. Remission accrued progressively and was frequently detectable only after two or more years, underscoring the importance of prolonged observation in real-world biologic studies.

Study 2 demonstrated that a multidimensional biological-cognitive approach integrating neuropsychological performance, body composition, metabolic profile, and inflammatory cytokine profiling significantly improves diagnostic discrimination along the neurocognitive continuum in elderly outpatients. The progressive predictive model showed consistent discriminative gains from each biological domain, with the inflammatory cytokine component providing the largest biological increment precisely for the most clinically difficult early-stage comparisons. Unsupervised analyses revealed a biologically structured and clinically interpretable patient heterogeneity, with two dimensions capturing cognitive-inflammatory severity and affective-functional phenotype

respectively, and two discrete biological phenotypes mapping coherently onto clinical diagnostic categories.

Study 3 demonstrated that acute lifestyle changes imposed by pandemic-related lockdown reduced physical activity and increased caloric intake, were associated with worsening of anthropometric, biochemical, body-composition, and non-invasive liver disease measures in patients with NAFLD, alongside a higher rate of hepatocellular carcinoma occurrence. Body compartment alterations emerged as the main predictor of adverse outcomes during lockdown, independently of liver fibrosis stage, identifying body composition as a potentially useful tool for NAFLD-tailored risk stratification.

6.2 Metainflammation across three clinical domains: treatment efficacy, diagnostic precision, and disease outcomes

A central and recurring theme across the three studies is the role of metabolic status as a modifier, rather than simply a comorbidity, of chronic disease expression and outcomes. In severe asthma, obesity defined a clinically distinct patient phenotype with greater comorbidity burden, attenuated type-2 inflammatory biomarkers, and lower quality of life at baseline, yet these metabolic differences did not translate into differential biologic effectiveness. This finding suggests that severe asthma treated with targeted biologics, the inflammatory pathway addressed by therapy is sufficiently dominant to override the metabolic modulation of the inflammatory background. However, the study also highlights that BMI alone is a crude proxy for metabolic status, and future research should investigate whether obesity phenotypes defined by body composition and metabolic-inflammatory profiling identify subgroups with differential biologic responsiveness.

In the cognitive domain, the picture was more nuanced. The patients enrolled in Study 2 exemplify the complexity of the geriatric patient presenting to a memory clinic: far from being defined solely by their cognitive complaint, they carried a substantial burden of multimorbidity including metabolic, cardiovascular, and psychiatric conditions, alongside polypharmacy and functional decline. It is precisely within this clinically complex background that systemic inflammation emerged as the most consistent and biologically coherent biological determinant of neurocognitive differentiation. Standard cardiometabolic indices did not differentiate diagnostic categories cross-sectionally, while the

inflammatory signal, captured both through the cytokine panel and through peripheral markers of low-grade inflammatory activity such as elevated AST and the neutrophil-to-lymphocyte ratio, tracked meaningfully with diagnostic category across the neurocognitive spectrum. This finding reframes the metabolic contribution to cognitive decline away from classical cardiometabolic risk and toward a broader biological framework in which systemic inflammation operating through multiple peripheral pathways simultaneously, emerges as the central mechanism linking metabolic dysfunction, ageing biology, and neurocognitive deterioration.

In NAFLD, the metabolic contribution to disease outcomes was most direct and quantitatively striking. Pandemic-related lifestyle deterioration produced rapid and measurable shifts in body composition increased fat mass, reduced fat-free mass and body cell mass, that independently predicted HCC occurrence and advanced tumour stage at diagnosis, beyond established risk factors including liver stiffness. This positions body composition not merely as a descriptive parameter but as a dynamic biological risk modifier with direct prognostic implications.

6.3 Clinical and public health implications

The clinical implications of these findings are both immediate and translational. In severe asthma management, the demonstration that biologic therapy is equally effective regardless of BMI category provides reassurance that obese patients should not be denied or delayed access to biologic treatment on the basis of weight alone. At the same time, weight management should be pursued as a complementary strategy to potentially enhance remission rates and reduce the comorbidity burden that limits quality of life in obese asthmatic patients.

In geriatric cognitive medicine, the multidomain framework provides a practical and scalable model for enhancing diagnostic precision using routinely accessible tools. The identification of domain-specific biological contributors at each diagnostic transition suggests targeted therapeutic opportunities. The bio-cognitive profiling framework offers a conceptual tool for patient stratification beyond categorical diagnosis, enabling more personalised monitoring and intervention strategies.

In NAFLD clinical practice, the findings identify body composition monitoring as a prognostically informative tool that should be integrated into routine follow-up, particularly during periods of

lifestyle disruption. The observation that body compartment changes independently predicted HCC occurrence and advanced tumour stage supports a shift from BMI-based to composition-based risk stratification in NAFLD, with potential implications for screening intensity and preventive intervention targeting.

Collectively, these implications support a broader paradigm shift in chronic disease management: from single-disease, single-biomarker approaches toward integrated, metabolism-informed and inflammation-aware frameworks that account for the biological complexity of the multimorbid patient and the interdependence of chronic conditions across the life course.

6.4 Future research directions

The findings of this thesis suggest several directions of potential interest for future research. The systematic integration of metabolic and inflammatory profiling into chronic disease evaluation may reveal biologically distinct patient subgroups with differential disease trajectories and therapeutic responsiveness that current categorical classifications fail to capture. Longitudinal studies with repeated biological assessment are needed to establish whether the metabolic-inflammatory signatures identified here precede clinical deterioration and whether early phenotyping can guide pre-emptive intervention. The development of composite scores integrating body composition, inflammatory profiling, and metabolic status across disease contexts could provide a common biological framework for risk stratification in chronic multimorbidity. Finally, embedding such tools into routine clinical workflows through registries and point-of-care algorithms, would represent the translational step toward metabolism-informed, inflammation-aware chronic disease management at scale.

7. REFERENCES

[1.] Hacker, K. The Burden of Chronic Disease. *Mayo Clin. Proc. Innov. Qual. Outcomes* 8, 112–119 (2024), doi:10.1016/j.mayocpiqo.2023.08.005.

[2.] Bhattacharya, S., Heidler, P. & Varshney, S. Incorporating neglected non-communicable diseases into the national health program, A review. *Front. Public Health* 10, (2023), doi:10.3389/fpubh.2022.1093170.

[3.] Bauer, U., Briss, P., Goodman, R. & Bowman, B. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. *The Lancet* 384, 45–52 (2014), doi:10.1016/s0140-6736(14)60648-6.

[4.] Hay, S. I., Ong, K. L., Santomauro, D. F., *et al.* Burden of 375 diseases and injuries, risk-attributable burden of 88 risk factors, and healthy life expectancy in 204 countries and territories, including 660 subnational locations, 1990–2023: a systematic analysis for the Global Burden of Disease Study 2023. *The Lancet* 406, 1873–1922 (2025), doi:10.1016/S0140-6736(25)01637-X.

[5.] Hajat, C. & Stein, E. The global burden of multiple chronic conditions: A narrative review. *Prev. Med. Rep.* 12, 284–293 (2018), doi:10.1016/j.pmedr.2018.10.008.

[6.] Bennett, J. E., O'Driscoll, O. N., Stevens, G. A., *et al.* Benchmarking progress in non-communicable diseases: a global analysis of cause-specific mortality from 2001 to 2019. *The Lancet* 406, 1255–1282 (2025), doi:10.1016/S0140-6736(25)01388-1.

[7.] Naghavi, M., Kyu, H. H., A, B., *et al.* Global burden of 292 causes of death in 204 countries and territories and 660 subnational locations, 1990–2023: a systematic analysis for the Global Burden of Disease Study 2023. *The Lancet* 406, 1811–1872 (2025), doi:10.1016/S0140-6736(25)01917-8.

[8.] Danesh, K., Kolstad, J., Parker, W. & Spinnewijn, J. *The Chronic Disease Index: Analyzing Health Inequalities Over the Lifecycle*. w32577 http://www.nber.org/papers/w32577.pdf (2024) doi:10.3386/w32577.

[9.] Fortuna, D., Caselli, L., Romoli, M., *et al.* Patient-centred estimation of multimorbidity in chronic disease populations: a novel approach integrating global burden of disease metrics and healthcare administrative data. *Popul. Health Metr.* 23, (2025), doi:10.1186/s12963-025-00404-x.

[10.] Johnston, M. C., Crilly, M., Black, C., Prescott, G. J. & Mercer, S. W. Defining and measuring multimorbidity: a systematic review of systematic reviews. *Eur. J. Public Health* 29, 182–189 (2019), doi:10.1093/eurpub/cky098.

[11.] Marengoni, A., Roso-Llorach, A., Vetrano, D. L., *et al.* Patterns of Multimorbidity in a Population-Based Cohort of Older People: Sociodemographic, Lifestyle, Clinical, and Functional Differences. *J. Gerontol. Ser. A* glz137 (2019), doi:10.1093/gerona/glz137.

[12.] Langenberg, C., Hingorani, A. & Whitty, C. Biological and functional multimorbidity—from mechanisms to management. *Nat. Med.* 29, 1649–1657 (2023), doi:10.1038/s41591-023-02420-6.

[13.] Vetrano, D. L., Roso-Llorach, A., Fernández, S., *et al.* Twelve-year clinical trajectories of multimorbidity in a population of older adults. *Nat. Commun.* 11, 3223 (2020), doi:10.1038/s41467-020-16780-x.

[14.] Zazzara, M. B., Triolo, F., Biscetti, L., *et al.* Biomarkers of multimorbidity: A systematic review. *Ageing Res. Rev.* 112, 102870 (2025), doi:10.1016/j.arr.2025.102870.

[15.] Kunnath, A., Sack, D. & Wilkins, C. Relative predictive value of sociodemographic factors for chronic diseases among All of Us participants: a descriptive analysis. *BMC Public Health* 24, (2024), doi:10.1186/s12889-024-17834-1.

[16.] Skou, S., Mair, F., Fortin, M., *et al.* Multimorbidity. *Nat. Rev. Dis. Primer* 8, (2022), doi:10.1038/s41572-022-00376-4.

[17.] Harris, R. Epidemiology of Chronic Disease: Global Perspectives. https://consensus.app/papers/epidemiology-of-chronic-disease-global-perspectives-harris/08d31ca3788b5e6a91b1536ff447e247/ (2012).

[18.] Poku, B. Global health challenges of chronic diseases increase and its' associated morbidity and mortality. *Int. J. Soc. Sci. Econ. Res.* https://doi.org/10.46609/ijsser.2023.v08i03.009 (2023) doi:10.46609/ijsser.2023.v08i03.009.

[19.] Patwardhan, V., Gil, G. F., Arrieta, A., *et al.* Differences across the lifespan between females and males in the top 20 causes of disease burden globally: a systematic analysis of the Global Burden of Disease Study 2021. *Lancet Public Health* 9, e282–e294 (2024), doi:10.1016/S2468-2667(24)00053-7.

[20.] Airhihenbuwa, C., Tseng, T.-S., Sutton, V. & Price, L. Global Perspectives on Improving Chronic Disease Prevention and Management in Diverse Settings. *Prev. Chronic. Dis.* 18, (2021), doi:10.5888/pcd18.210055.

[21.] Furman, D., Campisi, J., Verdin, E., *et al.* Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832 (2019), doi:10.1038/s41591-019-0675-0.

[22.] Cifuentes, M., Verdejo, H. E., Castro, P. F., *et al.* Low-Grade Chronic Inflammation: a Shared Mechanism for Chronic Diseases. *Physiology* 40, 4–25 (2025), doi:10.1152/physiol.00021.2024.

[23.] Libby, P., Smith, R., Rubin, E. J., *et al.* Inflammation unites diverse acute and chronic diseases. *Eur. J. Clin. Invest.* 54, e14280 (2024), doi:10.1111/eci.14280.

[24.] Hotamisligil, G. S. Inflammation, metaflammation and immunometabolic disorders. *Nature* 542, 177–185 (2017), doi:10.1038/nature21363.

[25.] Lee, Y. S. & Olefsky, J. Chronic tissue inflammation and metabolic disease. *Genes Dev.* 35, 307–328 (2021), doi:10.1101/gad.346312.120.

[26.] Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* 444, 860–867 (2006), doi:10.1038/nature05485.

[27.] Abtin, S., Ziveh, T. & Rezaee-Tavirani, M. The complicated relationship between inflammation and metabolic dysfunction. *J. Diabetes Metab. Disord.* 24, 237 (2025), doi:10.1007/s40200-025-01729-y.

[28.] Choi, W., Woo, G. H., Kwon, T.-H. & Jeon, J.-H. Obesity-Driven Metabolic Disorders: The Interplay of Inflammation and Mitochondrial Dysfunction. *Int. J. Mol. Sci.* 26, 9715 (2025), doi:10.3390/ijms26199715.

[29.] Papaioannou, O., Karampitsakos, T., Barbayianni, I., *et al.* Metabolic Disorders in Chronic Lung Diseases. *Front. Med.* 4, 246 (2018), doi:10.3389/fmed.2017.00246.

[30.] Rani, V., Deep, G., Singh, R. K., Palle, K. & Yadav, U. C. S. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci.* 148, 183–193 (2016), doi:10.1016/j.lfs.2016.02.002.

[31.] Dong, H., Sun, Y., Nie, L., *et al.* Metabolic memory: mechanisms and diseases. *Signal Transduct. Target. Ther.* 9, 38 (2024), doi:10.1038/s41392-024-01755-x.

[32.] Cosentino, F., Verma, S., Ambery, P., *et al.* Cardiometabolic risk management: insights from a European Society of Cardiology Cardiovascular Round Table. *Eur. Heart J.* 44, 4141–4156 (2023), doi:10.1093/eurheartj/ehad445.

[33.] Dove, A., Shang, Y., Xu, W., *et al.* The impact of diabetes on cognitive impairment and its progression to dementia. *Alzheimers Dement.* 17, 1769–1778 (2021), doi:10.1002/alz.12482.

[34.] Dove, A., Marseglia, A., Shang, Y., *et al.* Cardiometabolic multimorbidity accelerates cognitive decline and dementia progression. *Alzheimers Dement.* alz.12708 (2022) doi:10.1002/alz.12708.

[35.] Tsoukalas, D., Sarandi, E., Fragoulakis, V., *et al.* Metabolomics-based treatment for chronic diseases: results from a multidisciplinary clinical study. *BMJ Nutr. Prev. Health* 7, 357–365 (2024), doi:10.1136/bmjnph-2024-000883.

[36.] Smit, C., De Hoogd, S., Brüggemann, R. J. M. & Knibbe, C. A. J. Obesity and drug pharmacology: a review of the influence of obesity on pharmacokinetic and pharmacodynamic

parameters. *Expert Opin. Drug Metab. Toxicol.* 14, 275–285 (2018), doi:10.1080/17425255.2018.1440287.

[37.] Dostalek, M., Akhlaghi, F. & Puzanovova, M. Effect of Diabetes Mellitus on Pharmacokinetic and Pharmacodynamic Properties of Drugs. (2012).

[38.] Pillinger, T., McCutcheon, R. A., Vano, L., *et al.* Comparative effects of 18 antipsychotics on metabolic function in patients with schizophrenia, predictors of metabolic dysregulation, and association with psychopathology: a systematic review and network meta-analysis. *Lancet Psychiatry* 7, 64–77 (2020), doi:10.1016/S2215-0366(19)30416-X.

[39.] Guerra, J. V. S., Dias, M. M. G., Brilhante, A. J. V. C., *et al.* Multifactorial Basis and Therapeutic Strategies in Metabolism-Related Diseases. *Nutrients* 13, 2830 (2021), doi:10.3390/nu13082830.

[40.] Guo, H., Yang, J., Huang, J., *et al.* Comparative efficacy and safety of GLP-1 receptor agonists for weight reduction: A model-based meta-analysis of placebo-controlled trials. *Obes. Pillars* 13, 100162 (2025), doi:10.1016/j.obpill.2025.100162.

[41.] Nath, M., Bhattacharjee, K. & Choudhury, Y. Pleiotropic effects of anti-diabetic drugs: A comprehensive review. *Eur. J. Pharmacol.* 884, 173349 (2020), doi:10.1016/j.ejphar.2020.173349.

[42.] Jayasooriya, S. M., Devereux, G., Soriano, J. B., *et al.* Asthma: epidemiology, risk factors, and opportunities for prevention and treatment. *Lancet Respir. Med.* 13, 725–738 (2025), doi:10.1016/S2213-2600(24)00383-7.

[43.] Koppelman, G. H., Pino-Yanes, M., Melén, E., *et al.* Genetic and environmental risk factors for asthma: towards prevention. *Lancet Respir. Med.* 13, 1011–1025 (2025), doi:10.1016/S2213-2600(25)00256-5.

[44.] Porsbjerg, C., Melén, E., Lehtimäki, L. & Shaw, D. Asthma. *The Lancet* 401, 858–873 (2023), doi:10.1016/S0140-6736(22)02125-0.

[45.] Schatz, M., Hsu, J.-W. Y., Zeiger, R. S., *et al.* Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J. Allergy Clin. Immunol.* 133, 1549–1556 (2014), doi:10.1016/j.jaci.2013.10.006.

[46.] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2024. Updated May 2024. Available from: www.ginasthma.org

[47.] Global Initiative for Asthma. Difficult-To-Treat & Severe Asthma in Adolescent and Adult Patients, v5.0, 2024. Available from: www.ginasthma.org/reports

[48.] Chung, K. F., Wenzel, S. E., Brozek, J. L., *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* 43, 343–373 (2014), doi:10.1183/09031936.00202013.

[49.] Lommatzsch, M. & Virchow, J. C. Asthma remission: a call for a globally standardised definition. *Lancet Respir. Med.* 13, 2–3 (2025), doi:10.1016/S2213-2600(24)00304-7.

[50.] Canonica, G. W., Bagnasco, D., Bondi, B., *et al.* SANI clinical remission definition: a useful tool in severe asthma management. *J. Asthma* 61, 1593–1600 (2024), doi:10.1080/02770903.2024.2376919.

[51.] McDowell, P. J., McDowell, R., Busby, J., *et al.* Clinical remission in severe asthma with biologic therapy: an analysis from the UK Severe Asthma Registry. *Eur. Respir. J.* 62, 2300819 (2023), doi:10.1183/13993003.00819-2023.

[52.] Israel, E., Wechsler, M. E., Jackson, D. J. & Moore, W. C. Anti-cytokine biologics for asthma in adults. *The Lancet* 406, 2282–2294 (2025), doi:10.1016/S0140-6736(25)01625-3.

[53.] Hansen, S., Baastrup Søndergaard, M., Von Bülow, A., *et al.* Clinical Response and Remission in Patients With Severe Asthma Treated With Biologic Therapies. *CHEST* 165, 253–266 (2024), doi:10.1016/j.chest.2023.10.046.

[54.] Tooba, R. & Wu, T. D. Obesity and asthma: A focused review. *Respir. Med.* 204, 107012 (2022), doi:10.1016/j.rmed.2022.107012.

[55.] Lommatzsch, M., Brusselle, G. G., Levy, M. L., *et al.* A2BCD: a concise guide for asthma management. *Lancet Respir. Med.* 11, 573–576 (2023), doi:10.1016/S2213-2600(22)00490-8.

[56.] Shackleford, A., Heaney, L. G., Redmond, C., McDowell, P. J. & Busby, J. Clinical remission attainment, definitions, and correlates among patients with severe asthma treated with biologics: a systematic review and meta-analysis. *Lancet Respir. Med.* 13, 23–34 (2025), doi:10.1016/S2213-2600(24)00293-5.

[57.] Perez-de-Llano, L., Scelo, G., Tran, T. N., *et al.* Exploring Definitions and Predictors of Severe Asthma Clinical Remission after Biologic Treatment in Adults. *Am. J. Respir. Crit. Care Med.* 210, 869–880 (2024), doi:10.1164/rccm.202311-2192OC.

[58.] Ryman, J. T. & Meibohm, B. Pharmacokinetics of Monoclonal Antibodies. *CPT Pharmacomet. Syst. Pharmacol.* 6, 576–588 (2017), doi:10.1002/psp4.12224.

[59.] Scott, H. A., Gibson, P. G., Garg, M. L. & Wood, L. G. Airway inflammation is augmented by obesity and fatty acids in asthma. *Eur. Respir. J.* 38, 594–602 (2011), doi:10.1183/09031936.00139810.

[60.] Al-Lawati, F., Al-Mubaihsi, S. M., Jayakrishnan, B., Rizvi, S. & Al-Abri, M. A. Obstructive sleep apnea in patients with severe asthma: Prevalence and association between severity and asthma control. *Ann. Thorac. Med.* 17, 118–123 (2022), doi:10.4103/atm.atm_375_21.

[61.] on Behalf of SANI, Senna, G., Guerriero, M., *et al.* SANI-Severe Asthma Network in Italy: a way forward to monitor severe asthma. *Clin. Mol. Allergy* 15, 9 (2017), doi:10.1186/s12948-017-0065-4.

[62.] Schatz, M., Kosinski, M., Yarlas, A. S., *et al.* The minimally important difference of the Asthma Control Test. *J. Allergy Clin. Immunol.* 124, 719-723.e1 (2009), doi:10.1016/j.jaci.2009.06.053.

[63.] Althoff, M. D., Ghincea, A., Wood, L. G., Holguin, F. & Sharma, S. Asthma and Three Colinear Comorbidities: Obesity, OSA, and GERD. *J. Allergy Clin. Immunol. Pract.* 9, 3877–3884 (2021), doi:10.1016/j.jaip.2021.09.003.

[64.] Matucci, A., Micheletto, C. & Vultaggio, A. Severe Asthma and Biologics: Managing Complex Patients. *J. Investig. Allergy Clin. Immunol.* 33, 168–178 (2023), doi:10.18176/jiaci.0856.

[65.] Brumpton, B. M., Camargo, C. A., Romundstad, P. R., *et al.* Metabolic syndrome and incidence of asthma in adults: the HUNT study. *Eur. Respir. J.* 42, 1495–1502 (2013), doi:10.1183/09031936.00046013.

[66.] on Behalf of SANI, Senna, G., Guerriero, M., *et al.* SANI-Severe Asthma Network in Italy: a way forward to monitor severe asthma. *Clin. Mol. Allergy* 15, 9 (2017), doi:10.1186/s12948-017-0065-4.

[67.] Quanjer, P. H., Stanojevic, S., Cole, T. J., *et al.* Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur. Respir. J.* 40, 1324–1343 (2012), doi:10.1183/09031936.00080312.

[68.] Ten Have, L., Visser, E., Meulmeester, F. L., *et al.* Long-Term Weight Changes After Starting Anti–IL-5/5Ra Biologics in Severe Asthma: The Role of Oral Corticosteroids. *J. Allergy Clin. Immunol. Pract.* 11, 2748-2756.e3 (2023), doi:10.1016/j.jaip.2023.06.054.

[69.] Livingston, G., Huntley, J., Liu, K. Y., *et al.* Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. *The Lancet* 404, 572–628 (2024), doi:10.1016/S0140-6736(24)01296-0.

[70.] Reuben, D. B., Kremen, S. & Maust, D. T. Dementia Prevention and Treatment: A Narrative Review. *JAMA Intern. Med.* 184, 563 (2024), doi:10.1001/jamainternmed.2023.8522.

[71.] *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. (American psychiatric association, Washington, 2013).

[72.] Liu, Y., Tan, Y., Zhang, Z., *et al.* The interaction between ageing and Alzheimer's disease: insights from the hallmarks of ageing. *Transl. Neurodegener.* 13, 7 (2024), doi:10.1186/s40035-024-00397-x.

[73.] Abdul Manap, A. S., Almadodi, R., Sultana, S., *et al.* Alzheimer's disease: a review on the current trends of the effective diagnosis and therapeutics. *Front. Aging Neurosci.* 16, 1429211 (2024), doi:10.3389/fnagi.2024.1429211.

[74.] Rafii, M. S. & Aisen, P. S. Detection and treatment of Alzheimer's disease in its preclinical stage. *Nat. Aging* 3, 520–531 (2023), doi:10.1038/s43587-023-00410-4.

[75.] Mankhong, S., Kim, S., Lee, S., *et al.* Development of Alzheimer's Disease Biomarkers: From CSF- to Blood-Based Biomarkers. *Biomedicines* 10, 850 (2022), doi:10.3390/biomedicines10040850.

[76.] Petersen, R. C. Mild Cognitive Impairment. *N. Engl. J. Med.* 364, 2227–2234 (2011), doi:10.1056/NEJMcp0910237.

[77.] Calsolaro, V., Femminella, G. D., Rogani, S., *et al.* Behavioral and Psychological Symptoms in Dementia (BPSD) and the Use of Antipsychotics. *Pharm. Basel Switz.* 14, 246 (2021), doi:10.3390/ph14030246.

[78.] Lee, H. H., Chinnameyyappan, A., Feldman, O. J., *et al.* Behavioral and Psychological Symptoms (BPSD) in Alzheimer's Disease (AD): Development and Treatment. *Curr. Top. Behav. Neurosci.* 69, 245–273 (2025), doi:10.1007/7854_2024_566.

[79.] Alzheimer's Disease International. https://www.alzint.org/

[80.] Fabrizi, E., Ancidoni, A., Locuratolo, N., *et al.* The Italian guideline on diagnosis and treatment of dementia and mild cognitive impairment. *Age Ageing* 53, afae250 (2024), doi:10.1093/ageing/afae250.

[81.] Mehta, R. I. & Schneider, J. A. Neuropathology of the Common Forms of Dementia. *Clin. Geriatr. Med.* 39, 91–107 (2023), doi:10.1016/j.cger.2022.07.005.

[82.] Zeng, J., Hu, K., Wang, Z., *et al.* Cognitive Dysfunction and Dementia in Type 2 Diabetes Mellitus: Insights into Mechanisms, Models, and Therapeutics. *ACS Pharmacol. Transl. Sci.* 8, 2337–2352 (2025), doi:10.1021/acsptsci.5c00086.

[83.] Sangineto, M., Ciarnelli, M., Cassano, T., *et al.* Metabolic reprogramming in inflammatory microglia indicates a potential way of targeting inflammation in Alzheimer's disease. *Redox Biol.* 66, 102846 (2023), doi:10.1016/j.redox.2023.102846.

[84.] Fitzpatrick, A. L., Kuller, L. H., Lopez, O. L., *et al.* Midlife and Late-Life Obesity and the Risk of Dementia. *ARCH NEUROL* 66, (2009). doi: https://doi.org/10.1001/archneurol.2008.582.

[85.] Weintraub, S. Neuropsychological Assessment in Dementia Diagnosis. *Continuum* 28, 781–799 (2022), doi:10.1212/CON.0000000000001135.

[86.] Esteban-Cornejo, I., Ho, F. K., Petermann-Rocha, F., *et al.* Handgrip strength and all-cause dementia incidence and mortality: findings from the UK Biobank prospective cohort study. *J. Cachexia Sarcopenia Muscle* 13, 1514–1525 (2022), doi:10.1002/jcsm.12857.

[87.] Folstein, M. F., Folstein, S. E. & McHugh, P. R. Mini-Mental State Examination. doi:10.1037/t07757-000.

[88.] Horner, M. D., Teichner, G., Kortte, K. B. & Harvey, R. T. Construct Validity of the Babcock Story Recall Test. *Appl. Neuropsychol.* 9, 114–116 (2002), doi:10.1207/S15324826AN0902_7.

[89.] Constructional apraxia. in *Handbook of Clinical Neurology* vol. 151 331–348 (Elsevier, 2018)., doi:10.1016/B978-0-444-63622-5.00016-4.

[90.] Shao, Z., Janse, E., Visser, K. & Meyer, A. S. What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. *Front. Psychol.* 5, (2014), doi:10.3389/fpsyg.2014.00772.

[91.] Aprahamian, I., Martinelli, J. E., Neri, A. L. & Yassuda, M. S. The Clock Drawing Test A review of its accuracy in screening for dementia. *Dement. Neuropsychol.* 3, 74–80 (2009), doi:10.1590/S1980-57642009DN30200002.

[92.] Ott, B. R., Heindel, W. C., Whelihan, W. M., *et al.* Maze Test Performance and Reported Driving Ability in Early Dementia. *J. Geriatr. Psychiatry Neurol.* 16, 151–155 (2003), doi:10.1177/0891988703255688.

[93.] Appollonio, I., Leone, M., Isella, V., *et al.* The Frontal Assessment Battery (FAB): normative values in an Italian population sample. *Neurol. Sci.* 26, 108–116 (2005), doi:10.1007/s10072-005-0443-4.

[94.] Corrigan, J. D. & Hinkeldey, N. S. Relationships between Parts A and B of the Trail Making Test. *J. Clin. Psychol.* 43, 402–409 (1987), doi:10.1002/1097-4679(198707)43:4%3C402::AID-JCLP2270430411%3E3.0.CO;2-E.

[95.] Sala, S. D., Laiacona, M., Spinnler, H. & Ubezio, C. A cancellation test: its reliability in assessing attentional deficits in Alzheimer's disease. *Psychol. Med.* 22, 885–901 (1992), doi:10.1017/S0033291700038460.

[96.] Gasparini, M., Scandola, M., Amato, S., *et al.* Normative data beyond the total scores: a process score analysis of the Rey's 15 word test in healthy aging and Alzheimer's Disease. *Neurol. Sci.* 45, 2605–2613 (2024), doi:10.1007/s10072-024-07330-0.

[97.] Lawton, M. P. & Brody, E. M. Assessment of Older People: Self-Maintaining and Instrumental Activities of Daily Living. *The Gerontologist* 9, 179–186 (1969), doi:10.1093/geront/9.3_Part_1.179.

[98.] Katz, S. Assessing Self-maintenance: Activities of Daily Living, Mobility, and Instrumental Activities of Daily Living. *J. Am. Geriatr. Soc.* 31, 721–727 (1983), doi:10.1111/j.1532-5415.1983.tb03391.x.

[99.] Yesavage, J. A. & Sheikh, J. I. 9/Geriatric Depression Scale (GDS): Recent Evidence and Development of a Shorter Version. *Clin. Gerontol.* 5, 165–173 (1986), doi:10.1300/J018v05n01_09.

[100.] Cruz-Jentoft, A. J., Bahat, G., Bauer, J., *et al.* Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 48, 16–31 (2019), doi:10.1093/ageing/afy169.

[101.] Jessen, F., Amariglio, R. E., Van Boxtel, M., *et al.* A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement.* 10, 844–852 (2014), doi:10.1016/j.jalz.2014.01.001.

[102.] Petersen, R. C., Aisen, P., Boeve, B. F., *et al.* Mild cognitive impairment due to Alzheimer disease in the community. *Ann. Neurol.* 74, 199–208 (2013), doi:10.1002/ana.23931.

[103.] *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. (American psychiatric association, Washington, 2013).

[104.] Boyle, P. A., Buchman, A. S., Wilson, R. S., Leurgans, S. E. & Bennett, D. A. Association of Muscle Strength With the Risk of Alzheimer Disease and the Rate of Cognitive Decline in Community-Dwelling Older Persons. *Arch. Neurol.* 66, (2009), doi:10.1001/archneurol.2009.240.

[105.] Smith, A. D. & Refsum, H. Homocysteine, B Vitamins, and Cognitive Impairment. *Annu. Rev. Nutr.* 36, 211–239 (2016), doi:10.1146/annurev-nutr-071715-050947.

[106.] Chen, J., Liu, S., Wang, C., *et al.* Associations of Serum Liver Function Markers With Brain Structure, Function, and Perfusion in Healthy Young Adults. *Front. Neurol.* 12, 606094 (2021), doi:10.3389/fneur.2021.606094.

[107.] Galimberti, D., Fenoglio, C., Lovati, C., *et al.* Serum MCP-1 levels are increased in mild cognitive impairment and mild Alzheimer's disease. *Neurobiol. Aging* 27, 1763–1768 (2006), doi:10.1016/j.neurobiolaging.2005.10.007.

[108.] Westin, K., Buchhave, P., Nielsen, H., *et al.* CCL2 Is Associated with a Faster Rate of Cognitive Decline during Early Stages of Alzheimer's Disease. *PLoS ONE* 7, e30525 (2012), doi:10.1371/journal.pone.0030525.

[109.] Fakorede, S., Lateef, O. M., Garuba, W. A., *et al.* Dual impact of neuroinflammation on cognitive and motor impairments in Alzheimer's disease. *J. Alzheimers Dis. Rep.* 9, 25424823251341870 (2025), doi:10.1177/25424823251341870.

[110.] Mahapatra, G., Gao, Z., Bateman, J. R., *et al.* Blood-based bioenergetic profiling reveals differences in mitochondrial function associated with cognitive performance and Alzheimer's disease. *Alzheimers Dement.* 19, 1466–1478 (2023), doi:10.1002/alz.12731.

[111.] Tacke, F., Horn, P., Wai-Sun Wong, V., *et al.* EASL–EASD–EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J. Hepatol.* 81, 492–542 (2024), doi:10.1016/j.jhep.2024.04.031.

[112.] Powell, E. E., Wong, V. W.-S. & Rinella, M. Non-alcoholic fatty liver disease. *The Lancet* 397, 2212–2224 (2021), doi:10.1016/S0140-6736(20)32511-3.

[113.] Grander, C., Grabherr, F. & Tilg, H. Non-alcoholic fatty liver disease: pathophysiological concepts and treatment options. *Cardiovasc. Res.* 119, 1787–1798 (2023), doi:10.1093/cvr/cvad095.

[114.] Wong, V. W.-S., Ekstedt, M., Wong, G. L.-H. & Hagström, H. Changing epidemiology, global trends and implications for outcomes of NAFLD. *J. Hepatol.* 79, 842–852 (2023), doi:10.1016/j.jhep.2023.04.036.

[115.] Eurostat, Overweight and obesity - BMI statistics.https://ec.europa.eu/eurostat/statistics-explained

[116.] Khov, N. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. *World J. Gastroenterol.* 20, 6821 (2014), doi:10.3748/wjg.v20.i22.6821.

[117.] Lee, D. H. Imaging evaluation of non-alcoholic fatty liver disease: focused on quantification. *Clin. Mol. Hepatol.* 23, 290–301 (2017), doi:10.3350/cmh.2017.0042.

[118.] Alexander, M., Loomis, A. K., Van Der Lei, J., *et al.* Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. *BMC Med.* 17, 95 (2019), doi:10.1186/s12916-019-1321-x.

[119.] Tapper, E. B., Hunink, M. G. M., Afdhal, N. H., Lai, M. & Sengupta, N. Cost-Effectiveness Analysis: Risk Stratification of Nonalcoholic Fatty Liver Disease (NAFLD) by the Primary Care Physician Using the NAFLD Fibrosis Score. *PLOS ONE* 11, e0147237 (2016), doi:10.1371/journal.pone.0147237.

[120.] Vali, Y., Lee, J., Boursier, J., *et al.* Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study. *Lancet Gastroenterol. Hepatol.* 8, 714–725 (2023), doi:10.1016/S2468-1253(23)00017-1.

[121.] Sanyal, A. J., Van Natta, M. L., Clark, J., *et al.* Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease. *N. Engl. J. Med.* 385, 1559–1569 (2021), doi:10.1056/NEJMoa2029349.

[122.] Roeb, E. Excess Body Weight and Metabolic (Dysfunction)-Associated Fatty Liver Disease (MAFLD). *Visc. Med.* 37, 273–280 (2021), doi:10.1159/000515445.

[123.] Taheri, E., Bostick, R. M., Hatami, B., *et al.* Dietary and Lifestyle Inflammation Scores Are Inversely Associated with Metabolic-Associated Fatty Liver Disease among Iranian Adults: A Nested Case-Control Study. *J. Nutr.* 152, 559–567 (2022), doi:10.1093/jn/nxab391.

[124.] Lim, G.-Y., Chang, Y., Kim, I., *et al.* Long working hours and increased risks of lean non-alcoholic fatty liver disease among Korean men and women. *Sci. Rep.* 13, 12230 (2023), doi:10.1038/s41598-023-39154-x.

[125.] Dallio, M., Sangineto, M., Romeo, M., *et al.* Immunity as Cornerstone of Non-Alcoholic Fatty Liver Disease: The Contribution of Oxidative Stress in the Disease Progression. *Int. J. Mol. Sci.* 22, 436 (2021), doi:10.3390/ijms22010436.

[126.] Yaskolka Meir, A., Rinott, E., Tsaban, G., *et al.* Effect of green-Mediterranean diet on intrahepatic fat: the DIRECT PLUS randomised controlled trial. *Gut* 70, 2085–2095 (2021), doi:10.1136/gutjnl-2020-323106.

[127.] Ariya, M., Koohpayeh, F., Ghaemi, A., *et al.* Assessment of the association between body composition and risk of non-alcoholic fatty liver. *PLOS ONE* 16, e0249223 (2021), doi:10.1371/journal.pone.0249223.

[128.] Pickering, T. G., Hall, J. E., Appel, L. J., *et al.* Recommendations for Blood Pressure Measurement in Humans and Experimental Animals: Part 1: Blood Pressure Measurement in Humans: A Statement for Professionals From the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation* 111, 697–716 (2005), doi:10.1161/01.CIR.0000154900.76284.F6.

[129.] Neovius, M., Linné, Y. & Rossner, S. BMI, waist-circumference and waist-hip-ratio as diagnostic tests for fatness in adolescents. *Int. J. Obes.* 29, 163–169 (2005), doi:10.1038/sj.ijo.0802867.

[130.] Salgado, A. L. F. D. A., Carvalho, L. D., Oliveira, A. C., *et al.* Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arq. Gastroenterol.* 47, 165–169 (2010), doi:10.1590/S0004-28032010000200009.

[131.] Saunders, J. B., Aasland, O. G., Babor, T. F., De La Fuente, J. R. & Grant, M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption-II. *Addiction* 88, 791–804 (1993), doi:10.1111/j.1360-0443.1993.tb02093.x.

[132.] Boursier, J., Zarski, J.-P., De Ledinghen, V., *et al.* Determination of reliability criteria for liver stiffness evaluation by transient elastography. *Hepatology* 57, 1182–1191 (2013), doi:10.1002/hep.25993.

[133.] Sasso, M., Beaugrand, M., De Ledinghen, V., *et al.* Controlled Attenuation Parameter (CAP): A Novel VCTE^{TM} Guided Ultrasonic Attenuation Measurement for the Evaluation of Hepatic Steatosis: Preliminary Study and Validation in a Cohort of Patients with Chronic Liver Disease from Various Causes. *Ultrasound Med. Biol.* 36, 1825–1835 (2010), doi:10.1016/j.ultrasmedbio.2010.07.005.

[134.] Galle, P. R., Forner, A., Llovet, J. M., *et al.* EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J. Hepatol.* 69, 182–236 (2018), doi:10.1016/j.jhep.2018.03.019.

[135.] Lingiah, V. A., Niazi, M., Olivo, R., *et al.* Liver Transplantation Beyond Milan Criteria. *J. Clin. Transl. Hepatol.* 8, 1–7 (2020), doi:10.14218/JCTH.2019.00050.

[136.] Kaye, A. D., Cornett, E. M., Brondeel, K. C., *et al.* Biology of COVID-19 and related viruses: Epidemiology, signs, symptoms, diagnosis, and treatment. *Best Pract. Res. Clin. Anaesthesiol.* 35, 269–292 (2021), doi:10.1016/j.bpa.2020.12.003.

[137.] Zachary, Z., Brianna, F., Brianna, L., *et al.* Self-quarantine and weight gain related risk factors during the COVID-19 pandemic. *Obes. Res. Clin. Pract.* 14, 210–216 (2020), doi:10.1016/j.orcp.2020.05.004.

[138.] Fujii, H., Nakamura, N., Fukumoto, S., *et al.* Lifestyle changes during the coronavirus disease 2019 pandemic impact metabolic dysfunction–associated fatty liver disease. *Liver Int.* 42, 995–1004 (2022), doi:10.1111/liv.15158.

[139.] Azoulay, E., Yackobovitch-Gavan, M., Yaacov, H., *et al.* Weight Status and Body Composition Dynamics in Children and Adolescents During the COVID-19 Pandemic. *Front. Pediatr.* 9, 707773 (2021), doi:10.3389/fped.2021.707773.

[140.] Huang, D. Q., El-Serag, H. B. & Loomba, R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 18, 223–238 (2021), doi:10.1038/s41575-020-00381-6.

8. ACRONYMS

ACE-I	Angiotensin-converting enzyme inhibitor
ACQ	Asthma control questionnaire
ACT	Asthma control test
AD	Alzheimer's disease
ADL	Activities of daily living
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARIA	Amyloid-related imaging abnormalities
ASMI	Appendicular skeletal muscle index
AST	Aspartate aminotransferase
AUC	Area under the curve
AUDIT-C	Alcohol use disorders identification test
BCM	Body cell mass
BEC	Blood eosinophil count
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BPSD	Behavioural and psychological symptoms of dementia
cACLD	Compensated advanced chronic liver disease
CAP	Controlled attenuation parameter
CCB	Calciumchannel blockers
CCL3	C-C Motif Chemokine Ligand 3 (MIP-1α)
CCL4	C-C Motif Chemokine Ligand 4 (MIP-1β)
CDT	Clock drawing test
CRSsNP	Chronic rhinosinusitis without Nasal Polyps
CRSwNP	Chronic rhinosinusitis with nasal polyps
CS	Systemic corticosteroids
CT	Computer tomography
CXCL1	C-X-C Motif Chemokine Ligand 1 (GROα)
CXCL9	C-x-c motif chemokine ligand 9 (MIG)
DALY	Disability-adjusted life years
DAMP	Damage-associated molecular patterns
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic blood pressure
DPP4-I	Dipeptidylpeptidase inhibitors
DSAR	Danish severe asthma register
ECM	Extracellular mass
ED	Emergency department
EGF	Epidermal growth factor
eGFR	Estimated glomerular filtration rate
Eotaxin	Eotaxin (CCL11)
EPV	Events per variable
EWGSOP2	European Working Group on Sarcopenia in Older People 2
FAB	Frontal assessment battery

FeNO	Fractional exhaled nitric oxide
FEV1	Forced Expiratory Volume in 1 second
FFM	Free fat mass
FGF-2	Fibroblast growth factor 2
FIB-4	Fibrosis-4 index
FLT3L	FMS-like Tyrosine Kinase 3 Ligand
FM	Fat mass
fT3	Free triiodothyronine
fT4	Free tiroxine
FVC	Forced vital capacity
GBD	Global Burden of Disease
G-CSF	Granulocyte colony-stimulating factor
GDS-sf	Geriatric depression scale short form
GERD	Gastroesophageal reflux disease
GGT	Gamma-glutamyl transferase
GLP1	Glucagon-like peptide-1
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HbA1c	Glycated haemoglobin
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein
HLA	Human leukocyte antigen
HOMA-IR	Homeostasis model assessment of insulin resistance
HR	Hazard ratio
IADL	Instrumental activities of daily living
ICS	Inhaled corticosteroids
ICU	Intensive care unit
IFN	Interferon gamma
IFNA	Interferon alpha
IL	Interleukin
IL18R1	Interleukin-18 receptor 1
IL1F2	Interleukin-1 Beta (IL-1β)
ILC2	Innate lymphoid cells
IP-10	Interferon gamma-induced protein 10 (cxcl10)
IQR	Interquartile range
IRR	Incidence rate ratio
ISAR	International severe asthma registry
LABA	Long-acting beta-agonists
LDL	Low-density lipoprotein
LSM	Liver stiffness
MAMP	Metabolism-associated molecular patterns
MASH	Metabolic dysfunction–associated steatohepatitis
MASLD	Metabolic associated steatotis liver diseases
MBI	Mild behavioural impairment
MCI	Mild cognitive impairment
MCP-1	Monocyte chemoattractant protein-1 (ccl2)
MCP-3	Monocyte chemoattractant protein-3 (ccl7)

M-CSF	Macrophage colony-stimulating factor
MDC	Macrophage-derived chemokine (ccl22)
MDS	Multidimensional scaling
Met-ALD	Metabolic dysfunction-associated and alcohol-related liver disease
METAVIR	Meta-analysis of Histological Data in Viral Hepatitis
MICE	Multiple imputation by chained equations
mMCI	Mild cognitive impairment multiple domain
MMSE	Mini-mental state examination
MoCA	Montreal cognitive assessment
MRE	Magnetic resonance elastography
NAFLD	Non-alcoholic fatty liver diseases
NCD	Neurocognitive disorder
NCDs	Non-communicable diseases
NFS	NAFLD fibrosis score
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NKSF	Natural killer cell stimulatory factor (il-12)
NLPR3	Nod-like receptor protein 3
NLR	Neutrophil-to-Lymphocyte Ratio
OCR	Oxygen consumption rate
OR	Odds ratio
OSAS	Obstructive sleep apnea
PAMP	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PCA	Principal component analysis
PEF	Peak expiratory flow
PET	Positron emission tomography
PDGF	Platelet-derived growth factor
PLR	Platelet-to-Lymphocyte Ratio
PLT	Platelets
PSEN	Presenilin-1
RCT	Random clinical trial
ROC	Receiver Operating Characteristic curve
ROS	Reactive oxygen species
SANI	Severe asthma network Italy
SASP	Senescence-associated secretory phenotype
SBP	Systolyc blood pressure
SCC	Subjective memory complaints
sCD40L	Soluble CD40 ligand
SD	Standard deviation
SES	Socio-economic status
SGLT2	Sodium-glucose co-transporter 2
sMCI	Mild cognitive impairment single domain
SMM	Skeletal muscle mass
SMMI	Skeletal muscle mass index
SPM	Specialised pro-resolving mediators
T2DM	Type 2 diabetes mellitus

TBW	Total body water
TGFA	Transforming growth factor alpha
Th2	T-helper 2
TMT-A	Train making test A
TMT-B	Train making test B
TNF	Tumour necrosis factor
TNFB	Tumor necrosis factor beta (lymphotoxin-alpha)
TSH	Thyroid-stimulating hormone
TSLP	Thymic stromal lymphopoietin
UKSAR	Uk severe asthma registry
VCTE	Vibration-controlled transient elastography
VEGF-A	Vascular endothelial growth factor a
WBC	White blood cell count
WHR	Waist-to-hip ratio
YLD	Years Lived with Disability
YLL	Years of Life Lost
ZINB	Zero-inflated negative binomial

9. LIST OF FIGURES

Figure 24. Hepatocellular carcinoma occurrence in the period preceding the lockdown and during the lockdown.

Figure 25. Body composition variables and relative association with HCC occurrence during the lockdown (A) and HCC staged Milan-out criteria at diagnosis during lockdown (B).

10. LIST OF TABLES

Study 1.

Study 2.

Study 3.

11. PUBLICATIONS

1. **The influence of acute lifestyle changes on NAFLD evolution in a multicentre cohort: a matter of body composition.**
   Dallio M., Sangineto M., Romeo M., Cipullo M., Coppola A., Mammone S., **Di Gioia G.**, Masarone M., Persico M., Serviddio G., Federico A., *Nutrition and Diabetes*, 2024, doi:1413310.1038/s41387-024-00294-2

2. **Limits of pre-endoscopic scoring systems in geriatric patients with upper gastrointestinal bleeding.**
   Di Gioia G., Sangineto M., Paglia A., Cornacchia M.G., Parente F., Serviddio G., Romano A.D., Villani R., *Scientific Reports,* 2024, doi:1412022510.1038/s41598-024-70577-2

3. **Muscle health in the modern era of incretin-based therapies.**
   De Girolamo G., Sangineto M., Di Gioia G., Bianco R., Ciarnelli M., Serviddio G., *European Journal of Clinical Investigation,* 2026, doi:561e7015510.1111/eci.70155

4. **Comparative analysis of Sarcopenia in hospitalized elderly: exploring the impact of liver cirrhosis**.
   Romano A.D., Cornacchia M.G., Sangineto M., Di Gioia G., Villani R., Serviddio G., *Internal and Emergency Medicine*, 2024, doi:19719491957810.1007/s11739-024-03709-1

5. **Krebs cycle derivatives, dimethyl fumarate and itaconate, control metabolic reprogramming in inflammatory human microglia cell line.**
   Sangineto M., Ciarnelli M., Moola A., Naik Bukke V., Cassano T., Villani R., Romano A.D., **Di Gioia G.**, Avolio C., Serviddio G., *Mitochondrion,* 2024, doi:7910196610.1016/j.mito.2024.101966

6. **Impact of frailty on infection risk in non-transplant eligible multiple myeloma patients: a systematic review and meta-analysis.**
   Spataro F., Armentaro G., **Di Gioia G.**, Meloni P., Rossi I., Williams M., Romiti G.F., Talerico R., Villani R., Desantis V., Einsele H., Bencivenga L., Solimando A.G., *Leukemia,* 2026, doi:10.1038/s41375-026-02880-y

7. **High prevalence of false positive SARS-CoV2 serology in a cohort of patients with liver autoimmune diseases.**
   Cornacchia M.G., Sangineto M., Villani R., Cavallone F., **Di Gioia G.**, Cicciomessere P., Serviddio G., *Exploration of Medicine,* 2021, doi:24372377510.37349/emed.2021.00055