International PhD program in Cardiovascular Pathophysiology and Therapeutics

CardioPaTh
Cardiovascular
Pathophysiology
& Therapeutics

Senocardiology and cardiongevity – perspectives on AI-based precision longevity approaches in cardiology, cardiooncology and gender medicine in cardiovascular diseases.

PhD thesis

Ewelina Biskup (Evelyne Bischof), MD, MPH, FEFIM

Università degli Studi di Napoli Federico II
Cardiovascular Center OLV Aalst
INSELSPITAL Universitätsspital Bern Hôpital universitaire de Berne
Casa di Cura Montevergine – Alta specialità del cuore – Professionalità, dedizione e ricerca

Senocardiology and cardiongevity – perspectives on AI-based precision longevity approaches in cardiology, cardiooncology and gender medicine in cardiovascular diseases.

PhD thesis

Ewelina Biskup (Evelyne Bischof), MD, MPH, FEFIM

Promotor: Prof. Emanuele Barbato

University Federico II of Naples, Faculty of Medicine, Via Pansini n. 5, 80131 Naples, Italy

Co-promotor: Prof. Alex Zhavoronkov

John's Hopkins University, Buck Institute of Healthy Aging

Basel, 31/05/2023

Part I. Introduction to the thesis

Chapter 1. General introduction and outline of the thesis

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide, with an estimated 17.9 million deaths each year. Despite advances in medical knowledge and treatment, CVD continues to be a leading cause of death and disability, placing a significant burden on healthcare systems and societies around the world. One promising area of research that may help improve outcomes for patients with CVD is the use of artificial intelligence (AI) to develop precision approaches in cardiovascular longevity, including shaping of senocardiology and cardiolongevity.

AI-based precision longevity approaches involve the use of machine learning algorithms and other advanced computational techniques to analyze large datasets of medical information, including clinical data, medical images, and omics data. By analyzing this information, AI can help clinicians develop personalized prevention and treatment strategies that are tailored to individual patients' needs, improving outcomes and reducing healthcare costs. CUFII is a unique dataset including almost all of the multimodal, heterogenous datapoints. It was therefore a very suitable cohort for analysis of the biological age, which can help to personalize treatments, make individualized predictions, selecting the most effective medication or triaging patients.

Longevity medicine, largely driven by AI, should permeate and integrate with cardiology, oncology and other disciplines, including the subdiscipline of cardiooncology, in order to optimize the healthspan along the lifespan and to help prevent and manage the risks (prediction) of cardiovascular diseases, cardiovascular complications in cancer patients and . Cancer treatments, such as chemotherapy and radiation, can have significant cardiovascular side effects, increasing the risk of heart failure, arrhythmias, and other CVD complications. AI can help clinicians predict which patients are most likely to develop these complications, and tailor treatment plans to reduce the risk of cardiovascular complications. For example, AI can help clinicians monitor cardiac function during cancer treatment to detect early signs of cardiotoxicity, allowing for timely intervention to prevent further damage.

Sex and gender medicine has the potential to improve our understanding of gender-specific differences in the presentation, diagnosis, and treatment of all, especially the CVD. Women present with different symptoms of heart disease than men, or may be more likely to develop certain types of CVD, respond differently to strategic therapies and require a distinct preventative follow up regiment. AI can help identify these differences and tailor prevention and treatment strategies accordingly. By analyzing large datasets of medical information, AI can also help identify new risk factors for CVD in women and men, leading to more effective prevention and treatment strategies.

Despite the potential benefits of AI-based precision longevity approaches in cardiology, cardiooncology, and gender medicine, there are still many challenges that need to be addressed. One major challenge is the need for large, high-quality datasets that include diverse patient populations. Biobank are of great help, but those are limited in number and do not include longitudinal data on a specific disease cohort, such as the CUFII dataset, which is unique and valuable to identify core correlations not only between the pathology markers and

biological age, but also between longevity markers and the various pharmacotherapeutics and hospitalizations.

AI algorithms rely on large datasets to learn from and make accurate predictions. However, many medical datasets are limited in size or scope, or do not include diverse patient populations. This can limit the accuracy and applicability of AI-based models in clinical practice.

In this thesis, there are several thematic parts, divided by chapters, describing the general characteristics, as well as specific research conducted in the areas of cardiology, longevity medicine and the fusion – senocardiology and cariongevity, as well as sex and gender medicine in cardiology and longevity medicine, and finally oncology and longevity, including the new field of geroncology. All of these areas permeate each other and are composing a bigger entity, individualized advanced precision medicine, shaping alongside of the development and implementation of AI-based approaches. As these new arenas within established disciplines, as well as a new discipline (longevity medicime) is being shaped, research needs to implement concerns about the potential for AI algorithms reinforcing existing biases in medical care, or to perpetuate inequalities in access to care, such as via lacking sex and gender discrimination as biomarkers. Addressing these practical and strategic aspects will be critical to ensuring that AI-based precision longevity approaches are effective, equitable, and ethical.

The thesis covers a wide range of topics in the field of cardiology, longevity, geroncology, precision medicine, and artificial intelligence (AI) applications. It is structured into several parts and chapters, each addressing specific aspects of research and clinical practice.

In Part I, the thesis starts with an introduction that provides an overview of the entire work. It sets the stage for the subsequent chapters and highlights the importance of the topics covered. Part II focuses on the CUFII dataset, a longitudinal cardiopathy dataset, and explores its uniqueness. Descriptive analysis and core parameter correlations are thoroughly examined in Chapters 3 and 4, respectively. These chapters lay the foundation for the subsequent discussions on senocardiology, cardiongevity, and the application of AI in longevity medicine. Part III delves into senocardiology and cardiongevity, discussing the data on biological age as a predictor for cardiovascular disease. It also explores the relationship between biological and chronological age, the role of AI in longevity medicine, and AI applications in medicine in general. Geroncology and cardio-oncology take center stage in Part IV. Chapters 9 to 11 explore novel approaches in geroncology, including personalized precision cardio-oncology. The impact of biological age on decision-making in cardiological Basel and Baselland multicentric datasets is investigated in Chapter 12. Part V focuses on gender and biological sex as objective clinical variables. Chapters 13 and 14 shed light on the medical significance of gender and biological sex in internal medicine and cardiology, as well as the awareness of physicians regarding these dimensions. Precision medicine and its impact on gender and biological sex are discussed in Part VI. Chapters 15 to 17 highlight the importance of considering sex and gender in precision medicine, with a specific focus on medical oncology and the prism of COVID-19 pandemics. Basic research cardiology takes center stage in Part VII. Chapters 18 to 21 explore various aspects of myocardial function, microcirculation, IL-6 inhibitors, and the impact of stress, diet, and exercise on epigenetic age. Finally, Part VIII delves into precision senocardiology, focusing on AI-driven drug discovery and clinical trial settings. Chapters 22 to 24 discuss the potential of AI in

discovering new drugs, particularly in the context of COVID-19, and its applicability for drug repurposing.

In conclusion, this thesis provides a number of research projects focusing on various aspects of cardiology, longevity, geroncology, precision medicine, and AI applications. It explores unique datasets, correlations, and descriptive analyses while emphasizing the importance of biological age, gender, and biological sex in clinical practice. The thesis also highlights the potential of AI in drug discovery and clinical trial settings. Overall, this work aims to contribute to valuable insights to the field and opens avenues for further research and advancements in these areas.

Part II. CUFII dataset - longitudinal cardiopathy dataset and core correlations

Chapter 2. Dataset uniqueness.

The CUFII dataset is a unique large cohort with multidimensional, longitudinal parameters. It contains objective markers, critical to cardiovascular health and treatment trajectories. The uniquely rich dataset is well suited to identify crucial correlations between them and granular intersections towards objective functions, such as response to therapy. On a basic level, the dataset can be implemented in the conventional sick care aimed to understand the underlying factors that contribute to the response of specific medication, as well as to identify potential resistance-inducing risk factors in approaches of chronic heart failure. On another hand, since cardiovascular disease is a leading cause of death worldwide, and early detection and prevention are oftentimes not sufficient in order to reduce the number of deaths (Khan et al., 2020). Using the. Abundant dataset, the novel and tremendously important approach is to calculate the biological age of the patients and thus tackle the most personalized marker which correlates with age-related comorbidity and morbidity. Applying AI algorithms on the multimodal, heterogenic data ultimately helps to identify the most significant risk factors for cardiac morbidity and mortality, which helps to develop better prevention strategies and treatment plans to mitigate and eliminate these risks. The analysis of this data is further essential in helping to better discern and predict the risk factors associated with ever developing a cardiac pathology and to identify patients who are at a high risk, at low response to therapy etc. (Pothineni et al., 2020). By identifying these patients early, healthcare professionals can provide them with the necessary treatment and care, which can significantly reduce the risk of developing cardiovascular disease.

Chapter 3. Descriptive analysis.

Baseline Characters:

Age:

A total of 14091 patients had valid data of age in the study, with 2175 missing. The mean age was 71.71 (25-112) years old, standard error of mean was 0.11, median was 73 years old, standard deviation was 13.27, and the variance was 176.01. The 25th percentage was 63 years old, the 50th percentage was 73 years old, and the 75th was 81 years old.

Chronological age (as of 2023)		
N	Valid	14091
	Missing	2175
Mean		71.7083
Std. Error of Mean		.11176
Median		73.0000
Std. Deviation		13.2670
Variance		176.013
Range		87.00
Minimum		25.00
Maximum		112.00
Percentiles	25	63.0000
	50	73.0000
	75	81.0000

Table 1. Chronological age distribution of the CUFII dataset with standard deviations, variance, range and standard error calculations.

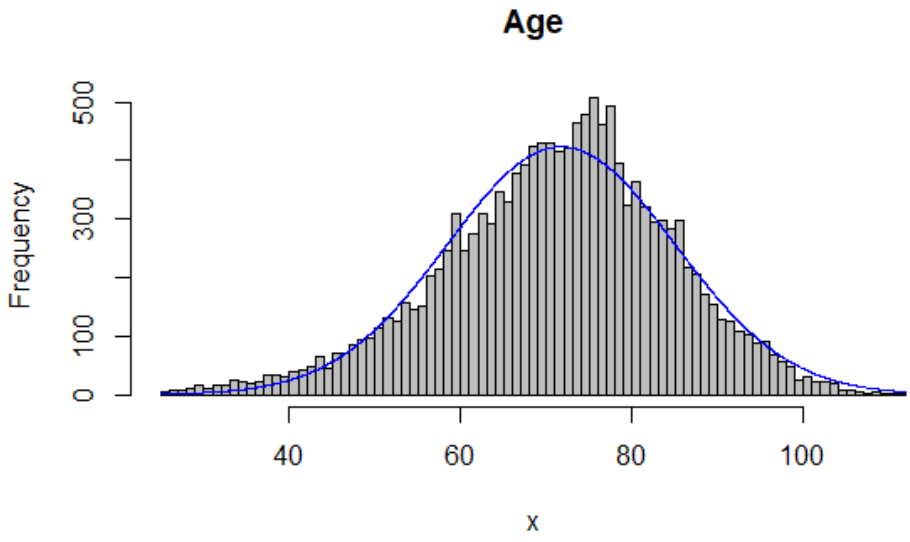


Figure 1. Chronological age distribution of the CUFII dataset.

Age-range					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	2	23	.1	.1	.1
	3	200	1.2	1.2	1.4
	4	563	3.4	3.4	4.8
	5	1533	9.4	9.4	14.2
	6	3245	19.9	19.9	34.1
	7	4529	27.7	27.7	61.8
	8	2859	17.5	17.5	79.3
	9	1049	6.4	6.4	85.7
	91	160	1.0	1.0	86.7
	miss	2175	13.3	13.3	100.0
	Total	16336	100.0	100.0	

Table 2. Age range of the CUFII dataset.

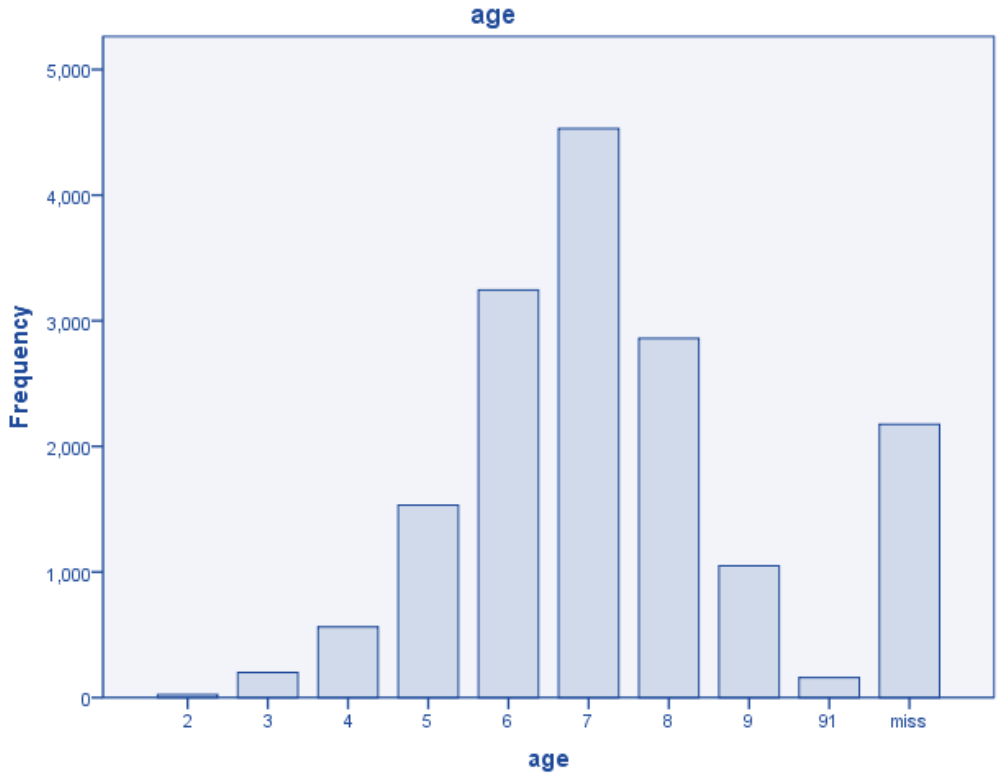


Figure 2. Frequency of chronological age distribution in the dataset.

Birth date

A total of 14161 patients had valid data of birth date in the study, with 2175 missing. The first was dated 01-JAN-1912 and the most latter was 12-SEP-1999.

Statistics		
DATADINASCITA		
N	Valid	14161
	Missing	2175
Minimum		01-JAN-1912
Maximum		12-SEP-1999

Table 3. Birth date distribution of the CUFII dataset.

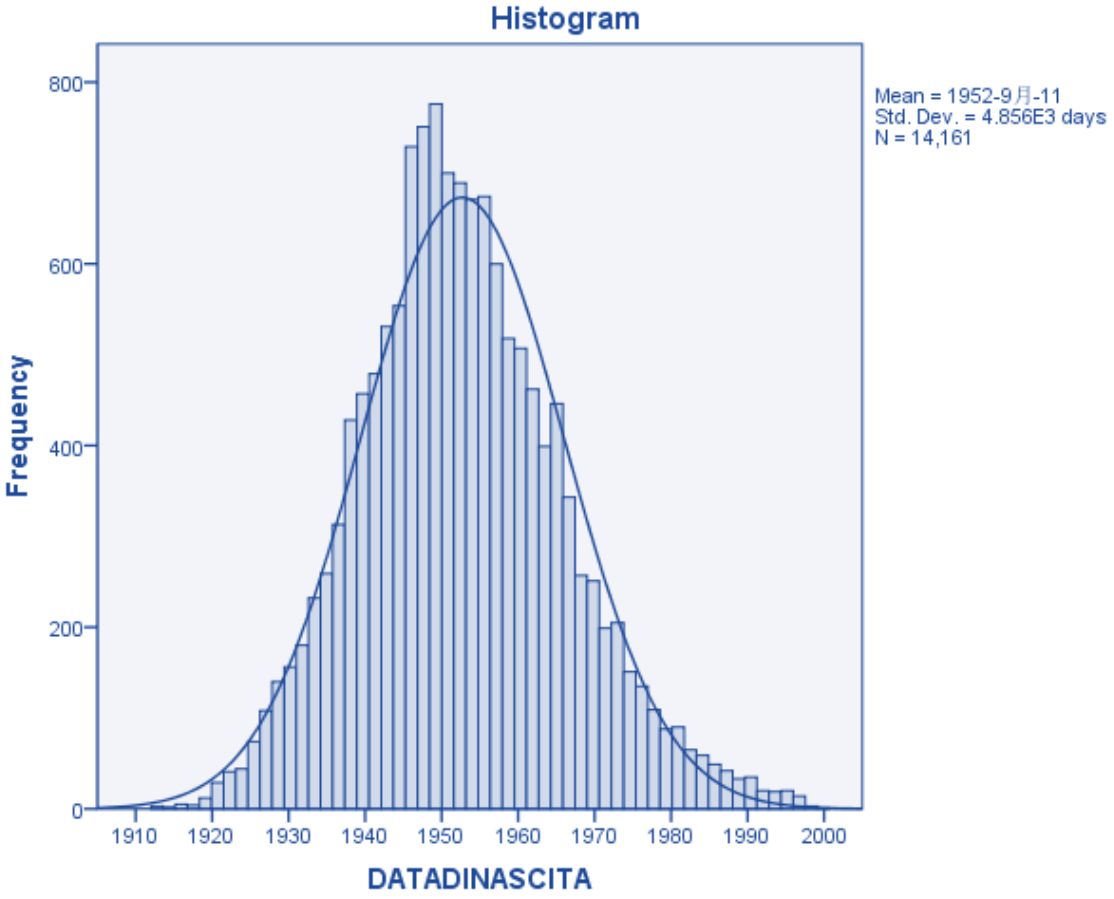


Figure 3. Birth date distribution of the CUFII dataset (DATADINASCITA – date of birth).

Death date

A total of 70 patients had valid data of death date in the study. The first was 01-JAN-1990 and the last was 05-FEB-2014.

Statistics		
DATA_DECESSO		
N	Valid	70
	Missing	16266
Minimum		01-JAN-1990
Maximum		05-FEB-2014

Table 4. Death date distribution of the CUFII dataset.

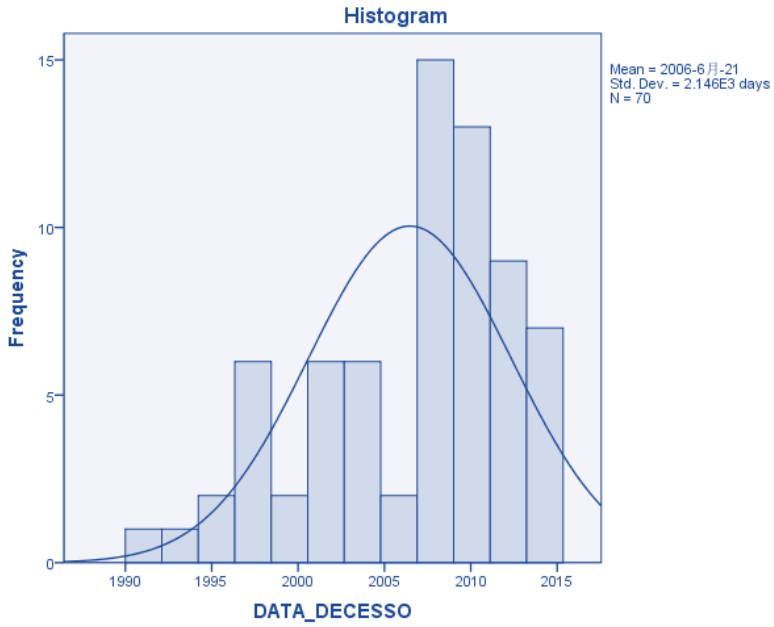


Figure 4. Death date distribution of the CUFII dataset.

Biological sex

A total of 14161 patients had valid data of gender in the study, with 2175 missing. There were 6246 females (38.2% in total) and 7915 males (48.5% in total).

Biological sex					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	6246	38.2	38.2	38.2
	2	7915	48.5	48.5	86.7
	miss	2175	13.3	13.3	100.0
	Total	16336	100.0	100.0	

1=female ; 2=male

Table 5. Sex proportion of the CUFII dataset.

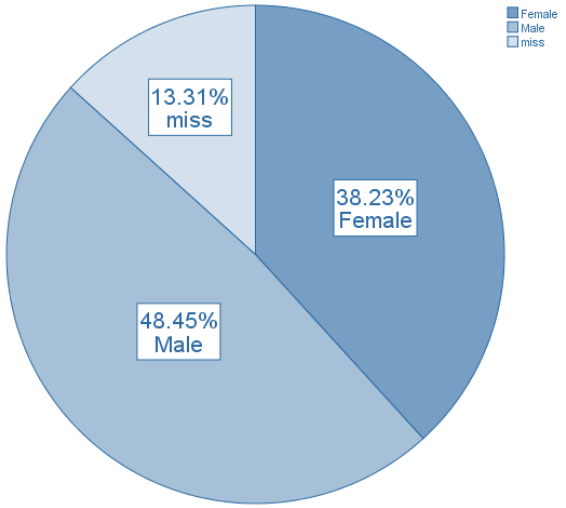


Figure 5. Sex proportion of the CUFII dataset.

Results with Biological Sex differetiation

Severity on carotid ultrasound

Of the patients who have normal carotid ultrasound, 3174 are male and 2507 are female. Of the patients who have mild atherosclerosis carotid artery, 6905 are male and 4674 are female. Of the patients who have moderate atherosclerosis carotid artery, 2164 are male and 1347 are female. Of the patients who have moderate atherosclerosis carotid artery, 5 are male and 2 are female. Sex is significantly correlated with the severity on carotid ultrasound (four categories) ($P<0.001$). Of the patients who have mild atherosclerosis at carotid ultrasound, 5606 are male and 4029 are female. Of the patients who have moderate atherosclerosis at carotid ultrasound, 1479 are male and 993 are female. Of the patients who have severe atherosclerosis at carotid ultrasound, 10 are male and 3 are female. Sex is not significantly correlated with the severity on carotid ultrasound (three categories) ($P=0.135$).

Events under pharmacotherapy

Significant correlation with male sex:

Of all the 2690 patients with use of calcium channel blocker "Ultimo_Diidropiridinici_Evento_TIA" data, 1643 were male and 1047 female, and use of a-channel blockers was significantly correlated with male sex.

Of all the 2983 patients with "DIIDROPIRIDINICI_COD_EVENTO_TIA" data, 1803 were male and 1180 female, and this was also significantly correlated with male sex.

Of all the 4100 patients with angiotensin converting enzyme inhibitors cod "ACEINIBITORI_COD" data, 2391 were male and 1709 female, and this event wa significantly correlated with male sex.

Of all the 3730 patients with cardiovascular therapy "total_therapy" data, 2179 are male and 1551 are female, and this event is significantly correlated with male sex.

Of all the 830 patients with hospitalization creatininemia "Ultima_Creatininemia" data, 610 are male and 220 are female, and this event is significantly correlated with male sex.

Of all the 221 patients with “Competing_CODE_FA” data, 138 are male and 83 are female, and this event is significantly correlated with male sex.

Of all the 442 patients with mean creatininine “MEAN_CREATININEMIA” data, 336 are male and 106 are female, and this event is significantly correlated with male sex.

Of all the 837 patients with last creatininie hard event “Ultima_Creatininemia_Evento_Hard” data, 617 are male and 220 are female, and this event is significantly correlated with male sex.

Of all the 446 patients with mean creatininine hard event “MEAN_CREATININEMIA_EVENTO_HARD” data, 341 are male and 105 are female, and this event is significantly correlated with male sex.

Of all the 829 patients with tia event last creatininine “Ultima_Creatininemia_Evento_TIA” data, 609 are male and 220 are female, and this event is significantly correlated with male sex.

Of all the 442 patients with tia event mean creatinine “MEAN_CREATININEMIA_EVENTO_TIA” data, 335 are male and 107 are female, and this event is significantly correlated with male sex.

Significant correlation with female sex:

Of all the 3086 patients with beta blocker therapy cod “BETABLOCCANTI_COD” data, 1482 were male and 1604 female, and this event is significantly correlated with female sex.

Of all the 2712 patients with last beta blocker “Ultimo_BETABLOCCANTI” data, 1312 were male and 1400 female, and this event was significantly correlated with female sex.

Of all the 2712 patients with “Ultimo_Betabloccanti_Evento_Hard” data, 1312 were male and 1400 are female, and this event was significantly correlated with female sex.

Of all the 3723 patients with angiotensin receptor blockers “AT1ANTAGONISTI__COD” data, 2085 were male and 1638 female, and this event was significantly correlated with female sex.

Of all the 3063 patients with beta blocker hard event “BETABLOCCANTI_COD_EVENTO_HARD” data, 1462 were male and 1601 female, and this event is significantly correlated with female sex.

Of all the 2712 patients with last beta blocker therapy “Ultimo_Betabloccanti_Evento_TIA” data, 1311 were male and 1401 female, and this event was significantly correlated with female sex.

Of all the 3084 patients with “BETABLOCCANTI_COD_EVENTO_TIA” data, 1481 are male and 1603 are female, and this event was significantly correlated with female sex.

Of all the 1748 patients with satin code “STATINE_COD” data, 896 were male and 852 female, and this event was significantly correlated with female sex.

Of all the 2155 patients with last statin “Ultimo_STATINE” data, 1144 were male and 1011 are female, and this event was significantly correlated with female sex.

Of all the 2122 patients with last statin hard event “Ultimo_Statine_Evento_Hard” data, 1114 were male and 1008 female, and this event is significantly correlated with female sex.

Of all the 1704 patients with “STATINE_COD_EVENTO_HARD” data, 858 are male and 846 are female, and this event was significantly correlated with female sex.

Of all the 2149 patients with “Ultimo_Statine_Evento_TIA” data, 1141 are male and 1008 are female, and this event is significantly correlated with female sex.

Of all the 1740 patients with “STATINE_COD_EVENTO_TIA” data, 891 are male and 849 are female, and this event is significantly correlated with female sex.

Of all the 4392 patients with hospitalization diuretici “Ultimo_DIURETICI” data, 2304 are male and 2088 are female, and this event is significantly correlated with female sex.

Of all the 4781 patients with hospitalization cod “DIURETICI__COD” data, 2457 are male and 2324 are female, and this event is significantly correlated with female sex.

Of all the 4382 patients with “Ultimo_Diuretici_Evento_Hard” data, 2296 are male and 2086 are female, and this event is significantly correlated with female sex.

Of all the 4767 patients with “DIURETICI_COD_EVENTO_HARD” data, 2449 are male and 2318 are female, and this event is significantly correlated with female sex.

Of all the 4396 patients with ultimo diuretici evento tia “Ultimo_Diuretici_Evento_TIA” data, 2307 are male and 2089 are female, and this event is significantly correlated with female sex.
Of all the 4772 patients with “DIURETICI_COD_EVENTO_TIA” data, 2450 are male and 2322 are female, and this event is significantly correlated with female sex.

BMIs
Of all patients, higher BMI (groups bmi_2, bmi_3, bmi_4, bmi_5, bmi_6, bmi_7, bmi_8, bmi_9, bmi_10, bmi_27) was significantly correlated with male sex.

CVDs
Of all patients, higher cardiovascular risk profile was significantly correlated with male sex.

Age
Higher age was significantly correlated with female sex.

Labs
Of all patients, GLICEMIA_1, GLICEMIA_2, GLICEMIA_3, GLICEMIA_4, GLICEMIA_5, GLICEMIA_6, GLICEMIA_7, GLICEMIA_8, GLICEMIA_9, GLICEMIA_10, GLICEMIA_11

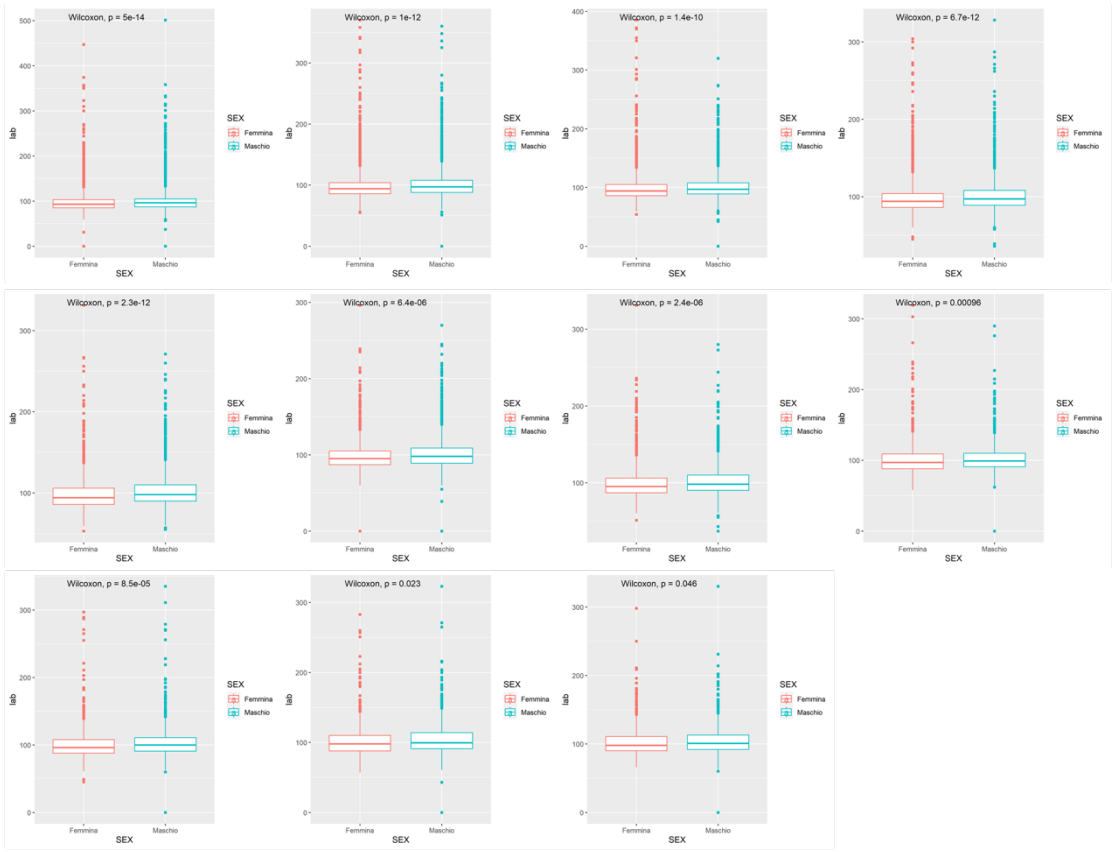


Figure 6. Biological sex and GLICEMIA.
Hyperglycemia is significantly correlated with male sex

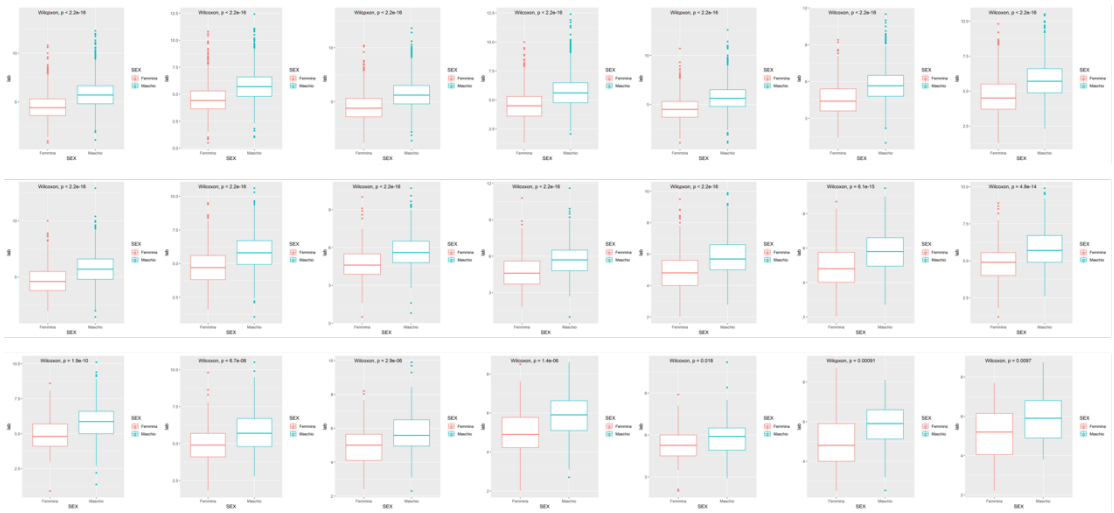


Figure 7. Biological sex and URICEMIA - significantly correlated with the mae sex.

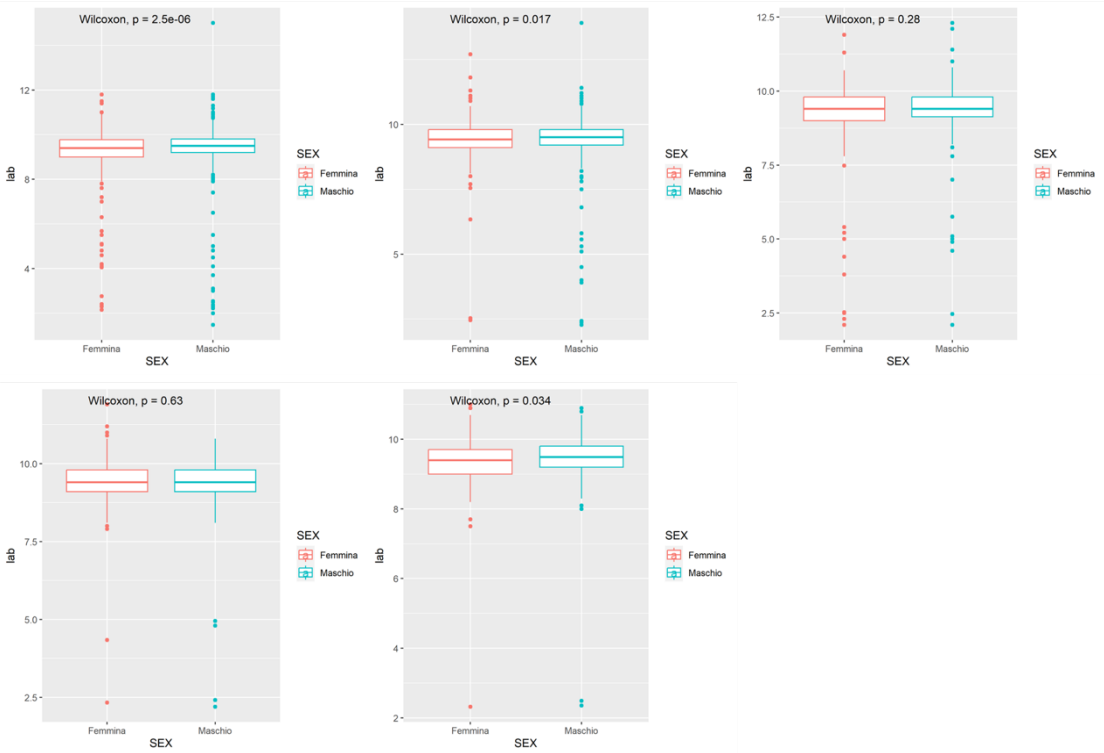


Figure 8. Biological sex and CALCEMIA - significantly correlated with male sex.

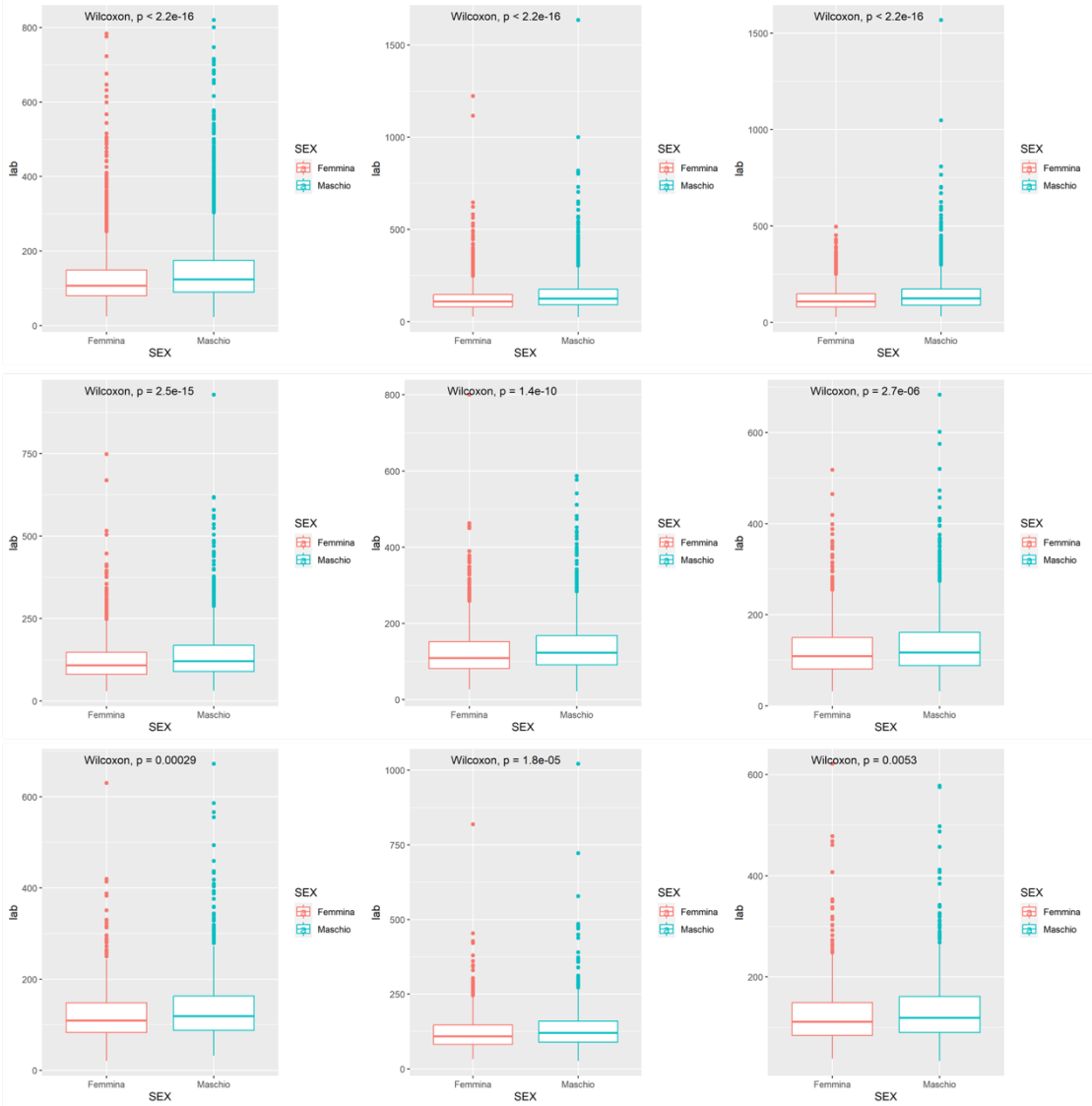


Figure 9. Biological sex and TRIGLICERIDI - significantly correlated with male sex.

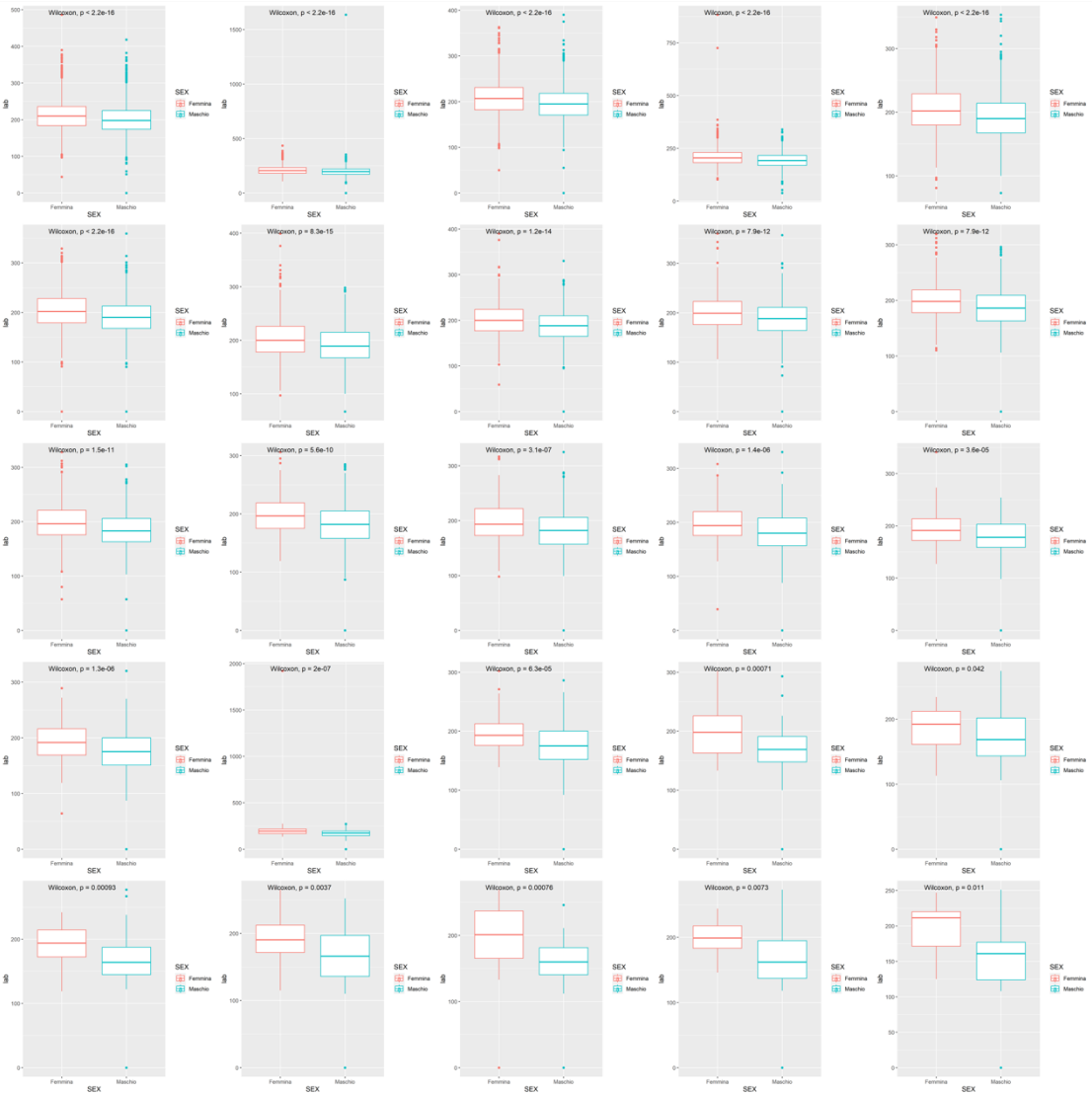


Figure 10. Biological sex and COLESTEROLO - significantly correlated with female sex.

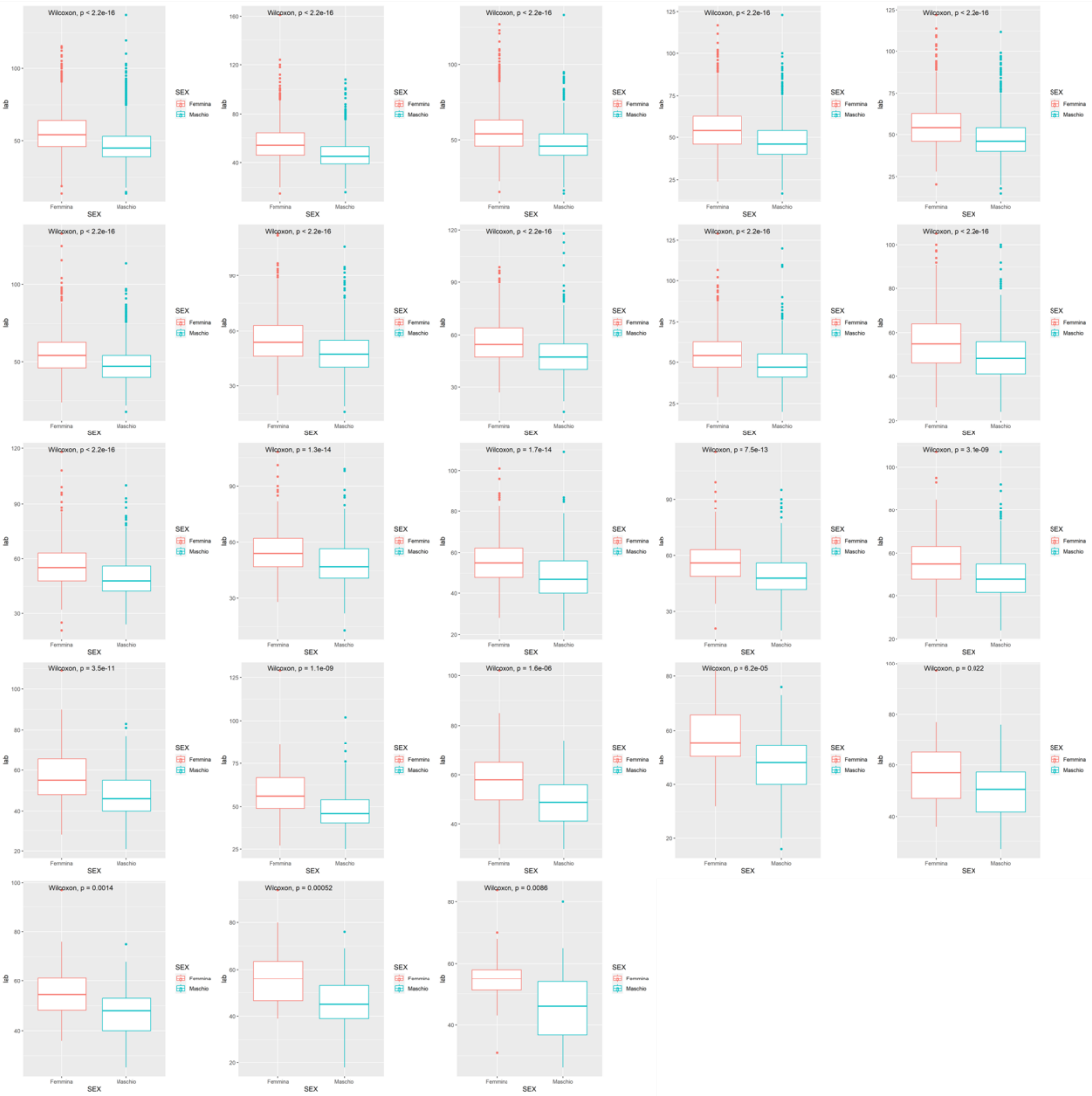


Figure 11. Biological sex and HDL - significantly correlated with female sex.

Advanced therapies

Of all patients, DIURETICI_1 to DIURETICI_18, DIURETICI_20 to DIURETICI_25, DIURETICI_27 to DIURETICI_32, DIURETICI_38 are significantly correlated with female sex. Of all patients, ALFABLOCCANTI_1 to ALFABLOCCANTI_17 are significantly correlated with male sex. Of all patients, BETABLOCCANTI_1 to BETABLOCCANTI_15 are significantly correlated with female sex. Of all patients, ACEINIBITORI_3 to ACEINIBITORI_15, ACEINIBITORI_18, ACEINIBITORI_19 are significantly correlated with male sex. Of all patients, DIIDROPIRIDINICI_1 to DIIDROPIRIDINICI_20 are significantly correlated with male sex. Of all patients, STATINE_1 to STATINE_6, STATINE_8 to STATINE_10, STATINE_12 to STATINE_15 are significantly correlated with female sex. Of all patients, INSULINA_5 is significantly correlated with female sex.

Cells

ESAMI_LABORATORIO.PROTEINE_TOTALI are significantly correlated with female sex. ESAMI_LABORATORIO.ALBUMINE are significantly correlated with male sex. ESAMI_LABORATORIO.GLOBULI_ROSSI are significantly correlated with female sex. ESAMI_LABORATORIO.GLOBULI_BIANCHI are significantly correlated with female sex. ESAMI_LABORATORIO.PIASTRINE are significantly correlated with female sex.

ESAMI_LABORATORIO.EMATOCRITO are significantly correlated with male sex. ESAMI_LABORATORIO.EMOGLOBINA are significantly correlated with male sex.

Other factors

Of all the 6021 patients with Troponin parameter data, 3853 are male and 2168 are female. Of all the 2978 patients with Baseline “AO_DILA_BASELINE_MAL” data, 1627 are male and 1351 are female. Of all the 2949 patients with Final “AO_DILA_FINAL_MAL” data, 1582 are male and 1367 are female.

Chapter 4. Correlations of core parameters.

Results based on Age

This file contains Age, Carotid ultrasound, Ultimo_Diidropiridinici_Evento_TIA, DIIDROPIRIDINICI_COD_EVENTO_TIA,AT1ANTAGONISTI__COD,ACEINIBITORI_COD,BETABLOCCANTI_COD,Ultimo_BETABLOCCANTI,Ultimo_Betabloccanti_Evento_Hard,BETABLOCCANTI_COD_EVENTO_HARD,Ultimo_Betabloccanti_Evento_TIA,BETABLOCCANTI_COD_EVENTO_TIA,STATINE_COD,Ultimo_STATINE,Ultimo_Statine_Evento_Hard,STATINE_COD_EVENTO_HARD,Ultimo_Statine_Evento_TIA,STATINE_COD_EVENTO_TIA,total_therapy,Competing_CODE_FA from 14161 patients first admission years 1980 to 2014.	This file contains Age, Carotid ultrasound, Last_Dihydropyridine_Event_TIA,DIHYDROPYRIDINE_COD_EVENT_TIA,AT1ANTAGONISTS__COD,ACEINHIBITORS_COD,BETA-BLOCKERS_COD,Last_BETA-BLOCKERS,Last_Beta-Blockers_Event_Hard,BETA-BLOCKERS_COD_EVENT_HARD,Last_TIA_Event_Beta-Blockers,TIA_EVENT_COD_BETA-BLOCKERS,COD_STATINS,Last_STATINS,Last_Statins_Event_Hard,STATINS_COD_EVENT_HARD,Last_Statins_Event_TIA,STATIN_COD_EVENT_TIA,total_therapy,Competing_CODE_FA from 14161 patients first admission years 1980 -2014.

Severity on carotid ultrasound

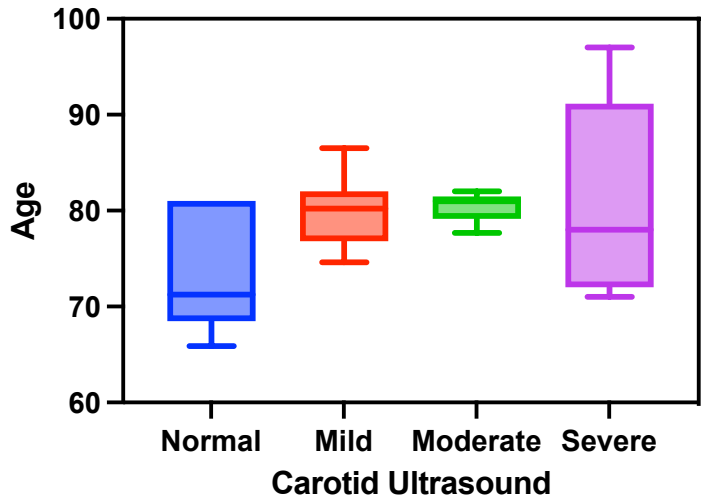


Figure 1. Age and severity on carotid ultrasound (four categories).
Of all the patients with data, patients who have normal carotid ultrasound are at a mean age of 73.60, patients who have mild atherosclerosis carotid artery are at a mean age of 79.58, patients who have moderate atherosclerosis carotid artery are at a mean age of 80.37, patients who have moderate atherosclerosis carotid artery are at a mean age of 80.87. Age is significantly correlated with the severity on carotid ultrasound (four categories) ($P<0.001$).

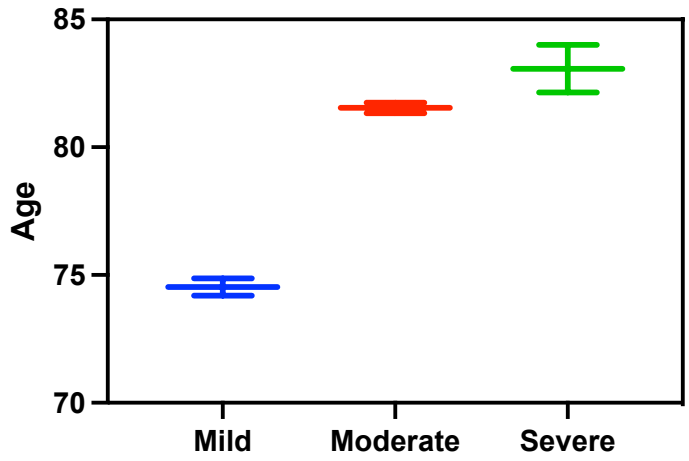


Figure 2. Age and severity on carotid ultrasound (three categories).
Of all the patients with data, patients who have mild atherosclerosis carotid artery are at a mean age of 74.53, patients who have moderate atherosclerosis carotid artery are at a mean age of 81.54, patients who have moderate atherosclerosis carotid artery are at a mean age of 83.07. Age is significantly correlated with the severity on carotid ultrasound (three categories) (P=0.008).

Events

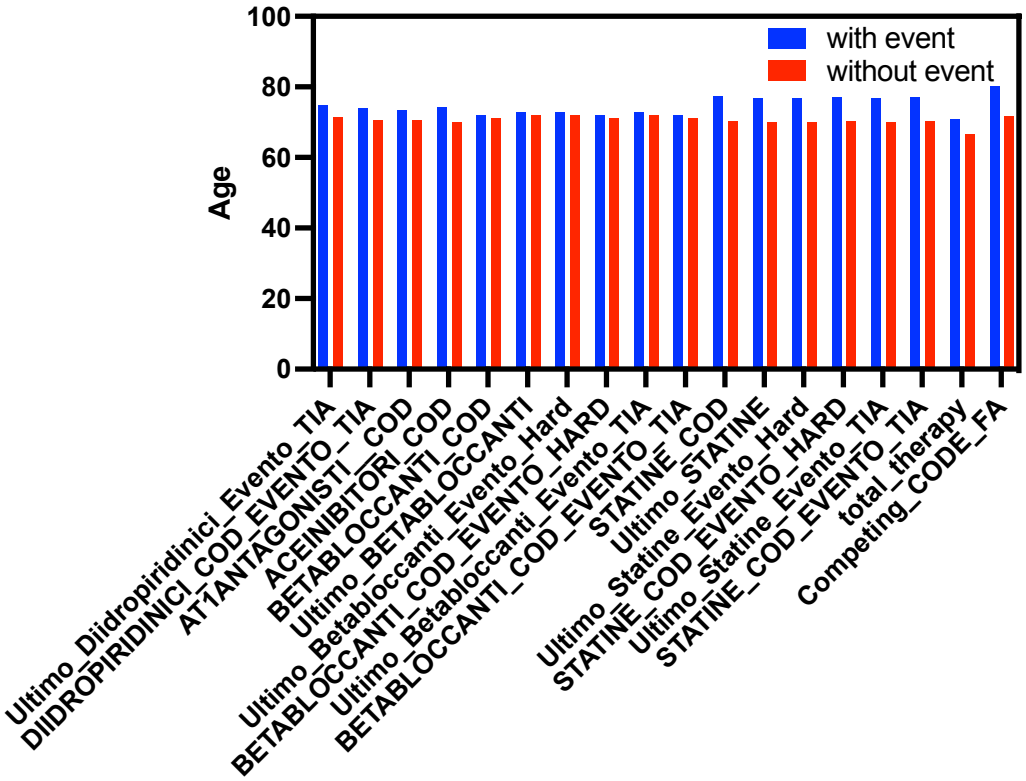


Figure 3. Age and events.

As shown in Fig 3., patients with use of calcium channel blocker "Ultimo_Diidropiridinici_Evento_TIA" are at a mean age of 74.00, without this event at a mean age of 71.17. This event is significantly correlated with age (P<0.001). Patients with "DIIDROPIRIDINICI_COD_EVENTO_TIA" are at a mean age of 74.59, without this event at a mean age of 70.53. This event is significantly correlated with age (P<0.001). Patients

with angiotensin receptor blockers code “AT1ANTAGONISTI__COD” are at a mean age of 73.19, without this event at a mean age of 70.57. This event is significantly correlated with age (P<0.001). Patients with angiotensin converting enzyme inhibitors cod “ACEINIBITORI_COD” are at a mean age of 74.22, without this event at a mean age of 70.01. This event is significantly correlated with age (P<0.001). Patients with beta blocker therapy cod “BETABLOCCANTI_COD” are at a mean age of 71.98, without this event at a mean age of 71.10. This event is significantly correlated with age (P<0.001). Patients with last beta blocker “Ultimo_BETABLOCCANTI” are at a mean age of 72.68, without this event at a mean age of 71.87. This event is significantly correlated with age (P=0.002). Patients with “Ultimo_Betabloccanti_Evento_Hard” are at a mean age of 72.69, without this event at a mean age of 71.86. This event is significantly correlated with age (P=0.002). Patients with beta blocker hard event “BETABLOCCANTI_COD_EVENTO_HARD” are at a mean age of 71.96, without this event at a mean age of 71.11. This event is significantly correlated with age (P<0.001). Patients with “Ultimo_Betabloccanti_Evento_TIA” are at a mean age of 72.68, without this event at a mean age of 71.87. This event is significantly correlated with age (P=0.002). Patients with “BETABLOCCANTI_COD_EVENTO_TIA” are at a mean age of 72.00, without this event at a mean age of 71.10.This event is significantly correlated with age (P<0.001). Patients with statin code “STATINE_COD” are at a mean age of 77.18, without this event at a mean age of 70.15. This event is significantly correlated with age (P<0.001). Patients with last statin “Ultimo_STATINE” are at a mean age of 76.77, without this event at a mean age of 69.97. This event is significantly correlated with age (P<0.001). Patients with last statin hard event “Ultimo_Statine_Evento_Hard” are at a mean age of 76.71, without this event at a mean age of 70.03. This event is significantly correlated with age (P<0.001). Patients with “STATINE_COD_EVENTO_HARD” are at a mean age of 77.18, without this event at a mean age of 70.15. This event is significantly correlated with age (P<0.001). Patients with tia event last statine “Ultimo_Statine_Evento_TIA” are at a mean age of 76.74, without this event at a mean age of 69.99. This event is significantly correlated with age (P<0.001). Patients with “STATINE_COD_EVENTO_TIA” are at a mean age of 77.16, without this event at a mean age of 70.16. This event is significantly correlated with age (P<0.001). Patients with cardiovascular therapy “total_therapy” are at a mean age of 70.85, without this event at a mean age of 66.624. This event is significantly correlated with age (P<0.001). Patients with “Competing_CODE_FA” at a mean age of 80.02, without this event at a mean age of 71.74. This event is significantly correlated with age (P<0.001).

Results based on BMI

This file contains BMI, Carotid ultrasound, Ultimo_Diidropiridinici_Evento_TIA,DIIDROPIRIDINICI_COD_EVENTO_TIA,AT1ANTAGONISTI__COD,ACEINIBITORI_COD,BETABLOCCANTI_COD,Ultimo_BETABLOCCANTI,BETABLOCCANTI_COD_EVENTO_HARD,Ultimo_Betabloccanti_Evento_TIA,BETABLOCCANTI_COD_EVENTO_TIA,GLICEMIA (from 1 to 103) ,CREATININEMIA (from 1 to 103) ,URICEMIA (from 1 to 103) ,CALCEMIA (from 1 to 103) ,TRIGLICERIDI (from 1 to 103) ,COLESTEROLO (from 1 to 103) ,HDL (from 1 to 103) ,DIURETICI (from 1 to 126) ,ALFABLOCCANTI (from 1 to 126) ,BETABLOCCANTI (from 1 to 126) ,ACEINIBITORI (from 1 to 126) ,DIIDROPIRIDINICI (from 1 to 126) ,STATINE (from 1 to 126) ,INSULINA (from 1 to 126),ESAMI_LABORATORIO.PROTEINE_TOTALI,ESAMI_LABORATORIO.ALBUMINE,ESAMI_LABORATORIO.GLOBULI_ROSSI,ESAMI_LABORATORIO.GLOBULI_BIANCHI,ESAMI_LABORATORIO.PIASTRINE,ESAMI_LABORATORIO.EMATOCRITO,ESAMI_LABORATORIO.EMOGLOBINA from 14161 patients first admission years 1980 to 2014.	This file contains BMI, Carotid ultrasound, Last_Dihydropyridine_Event_TIA,DIHYDROPYRIDINE_COD_EVENT_TIA,AT1ANTAGONISTS__COD,ACEINHIBITORS_COD,BETA-BLOCKERS_COD,Last_BETA-BLOCKERS,BETA-BLOCKERS_COD_EVENT_HARD,Last_Beta-Blockers_Event_TIA,BETABLOCCANTI_COD_EVENT_TIA,BLOOD GLUCOSE (from 1 to 103) ,CREATININEMIA (from 1 to 103) ,URICEMIA (from 1 to 103) , CALCAEMIA (from 1 to 103) ,TRIGLYCERIDES (from 1 to 103) ,CHOLESTEROL (from 1 to 103) ,HDL (from 1 to 103) ,DIURETICS (from 1 to 126) ,ALPHASE BLOCKERS (from 1 to 126) ,BETA BLOCKERS (from 1 to 126) ,ACEINHIBITORS (from 1 to 126) ,DIHYDROPYRIDINE (from 1 to 126) ,STATINS (from 1 to 126) ,INSULIN (from 1 to 126),LAB_TESTS.TOTAL PROTEINS,LAB_TESTS.ALBUMINS,LAB_TESTS.RED_BLOODS ,LABORATORY_EXAMS.WHITE_CELLS,LABORATORY_EXAMS.PLATELETS,LABORATORY_EXAMS.EMATOCRIT,LABORATORY_EXAMS.HEMOGLOBIN from 14161 patients first admission years 1980-2014.

Severity on carotid ultrasound

Of all the patients with data, patients who have normal carotid ultrasound are at a mean BMI of 27.10, patients who have mild atherosclerosis carotid artery are at a mean BMI of 25.34, patients who have moderate atherosclerosis carotid artery are at a mean BMI of 27.93, patients who have moderate atherosclerosis carotid artery are at a mean BMI of 29.64. BMI is significantly correlated with the severity on carotid ultrasound (four categories) (P<0.001)(Figure 4).

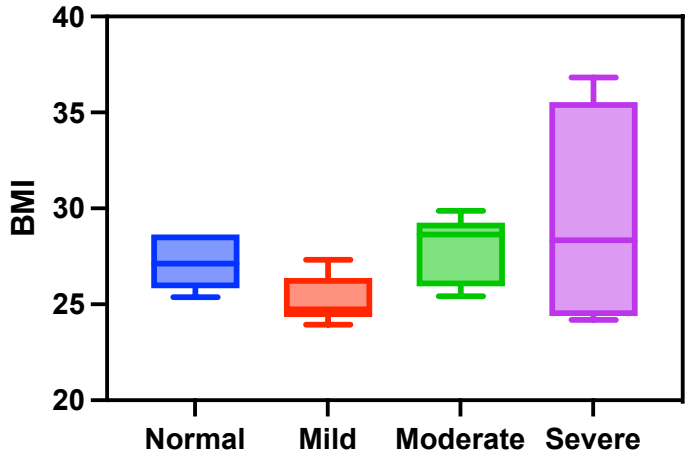


Figure 4. BMI and severity on carotid ultrasound (four categories).

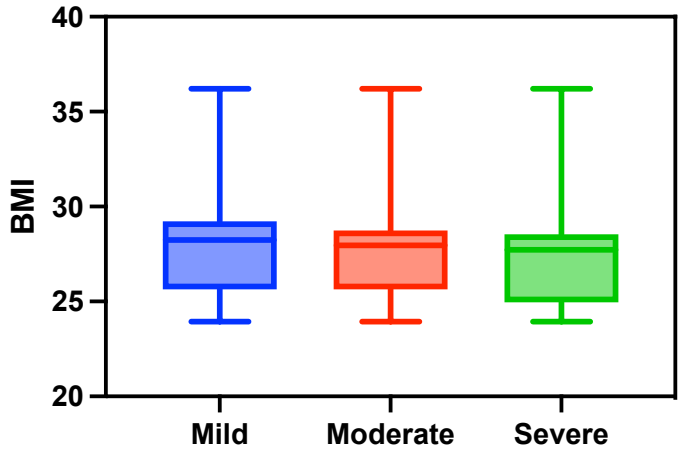


Figure 5. BMI and severity on carotid ultrasound (three categories).
Of all the patients with data, patients who have mild atherosclerosis carotid artery are at a mean BMI of 27.56, patients who have moderate atherosclerosis carotid artery are at a mean BMI of 27.20, patients who have moderate atherosclerosis carotid artery are at a mean BMI of 26.86. BMI is significantly correlated with the severity on carotid ultrasound (three categories) (P=0.507).

Events

The mean BMI of the patients with calcium channel blocker "Ultimo_Diidropiridinici_Evento_TIA" are 28.24, 28.21, 28.15, 28.31, 28.23, 28.24, 28.24,28.33, 28.28, 28.31, 28.42, 28.03, 28.05, 28.22, 28.12, 28.37, 28.97, 28.77, 28.48 in BMI_2 to BMI_16, BMI_28, BMI_29, BMI_31, BMI_32, and without this event are 27.66, 27.57, 27.54, 27.51, 27.40, 27.43 27.46, 27.51, 27.40, 27.50, 27.50, 27.30, 27.49, 27.64, 27.41, 26.70, 27.00, 26.33, 26.13. BMI_2 to BMI_16, BMI_28, BMI_29, BMI_31, BMI_32 are significantly correlated with use of calcium channel blocker "Ultimo_Diidropiridinici_Evento_TIA". The mean BMI of the patients with "DIIDROPIRIDINICI_COD_EVENTO_TIA" are 28.24, 28.20, 28.17, 28.25, 28.19, 28.23, 28.22, 28.38, 28.20, 28.41, 27.95, 28.21, 28.40, 8.41, 29.21, 29.27, 29.34, 29.83, 29.38, 29.54, 29.23, 29.23, 28.72, 29.59, 29.62, 29.75, 28.86, 29.58, 28.89 in BMI_2 to BMI_10, BMI_12, BMI_13, BMI_16, BMI_24 to BMI_40, and without this event are 27.77, 27.61, 27.57, 27.59, 27.47, 27.50, 27.54, 27.56, 27.52, 27.61, 27.43, 27.44, 27.11, 26.40, 26.77, 26.67, 26.33, 26.80, 26.31, 26.25, 26.16, 26.19, 26.12, 25.52, 26.08, 25.55, 25.18, 24.85, 24.90. BMI_2 to BMI_10, BMI_12, BMI_13, BMI_16, BMI_24 to BMI_40 are significantly correlated with "DIIDROPIRIDINICI_COD_EVENTO_TIA". The mean BMI of the patients with angiotensin receptor blockers code "AT1ANTAGONISTI__COD" are 28.35, 28.13, 28.05, 28.07, 27.92, 27.93, 27.97, 29.18, 29.26, 29.24, 29.99 in BMI_2 to BMI_8, BMI_26, BMI_27, BMI_32, BMI_35, and without this event are 27.68, 27.58, 27.55, 27.60, 27.52, 27.58, 27.59, 27.25, 27.02, 26.55, 25.86. BMI_2 to BMI_8, BMI_26, BMI_27, BMI_32, BMI_35 are significantly correlated with angiotensin receptor blockers code "AT1ANTAGONISTI__COD". The mean BMI of the patients with angiotensin converting enzyme inhibitors cod "ACEINIBITORI_COD" are 28.09, 27.98, 27.96, 27.97, 27.93, 27.99, 28.01, 28.06, 29.49 in BMI_2 to BMI_9, BMI_41, and without this event are 27.78, 27.64, 27.59, 27.64, 27.51, 27.53, 27.55, 27.64, 25.42. BMI_2 to BMI_9, BMI_41 is significantly correlated with angiotensin converting enzyme inhibitors cod "ACEINIBITORI_COD". The mean BMI of the patients with beta blocker therapy cod "BETABLOCCANTI_COD" is 28.04 in BMI_2, and without this event is 27.82. BMI_2 is significantly correlated with beta

blocker therapy cod “BETABLOCCANTI_COD”. The mean BMI of the patients with last beta blocker “Ultimo_BETABLOCCANTI” are 28.44, 28.85, 30.51, 29.84 in BMI_17, BMI_23, BMI_36, BMI_41, and without this event are 27.65, 27.56, 26.90, 25.82. BMI_17, BMI_23, BMI_36, BMI_41 is significantly correlated with last beta blocker “Ultimo_BETABLOCCANTI”. The mean BMI of the patients with beta blocker hard event “BETABLOCCANTI_COD_EVENTO_HARD” is 28.03 in BMI_2, and without this event is 27.83. BMI_2 is significantly correlated with beta blocker hard event “BETABLOCCANTI_COD_EVENTO_HARD”. The mean BMI of the patients with “Ultimo_Betabloccanti_Evento_TIA” are 28.46, 30.51, 29.84 in BMI_17, BMI_36, BMI_41, and without this event are 27.63, 26.90, 25.82. BMI_17, BMI_36, BMI_41 are significantly correlated with “Ultimo_Betabloccanti_Evento_TIA”. The mean BMI of the patients with “BETABLOCCANTI_COD_EVENTO_TIA” is 28.04 in BMI_2, and without this event is 27.82. BMI_2 is significantly correlated with “BETABLOCCANTI_COD_EVENTO_TIA”.

Labs

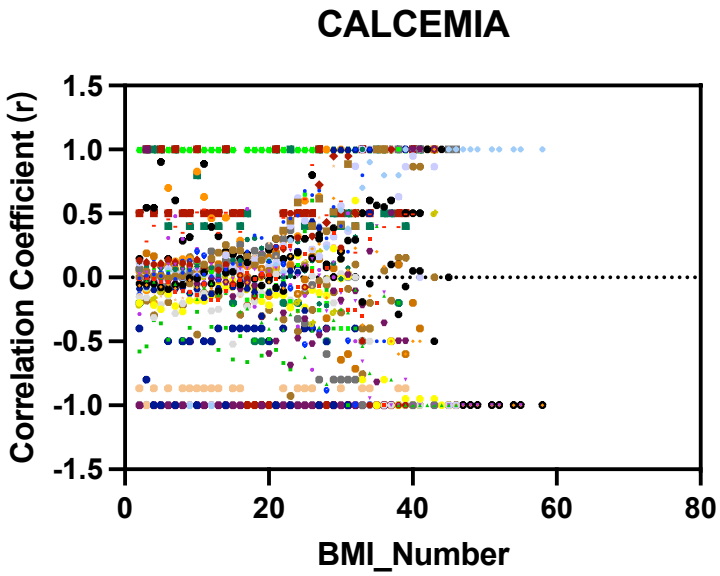


Figure 6 BMI and CALCEMIA.
BMI is not significantly correlated with CALCEMIA.

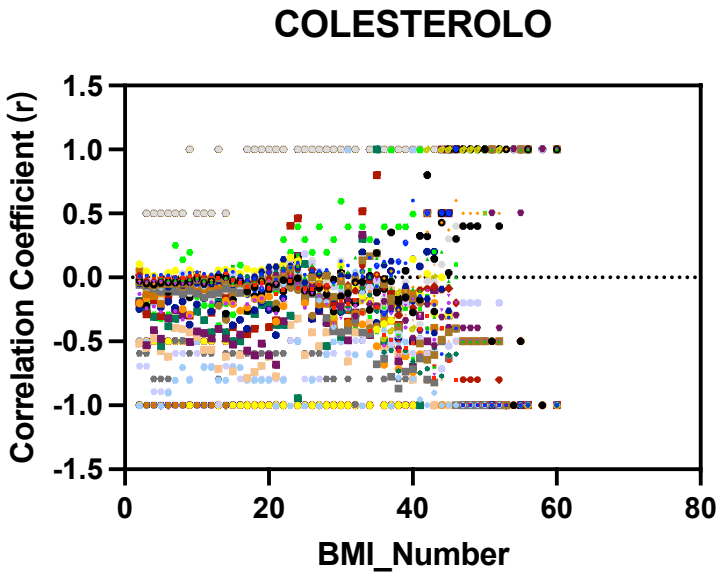


Figure 7. BMI and COLESTEROLO.
BMI is negatively correlated with COLESTEROLO.

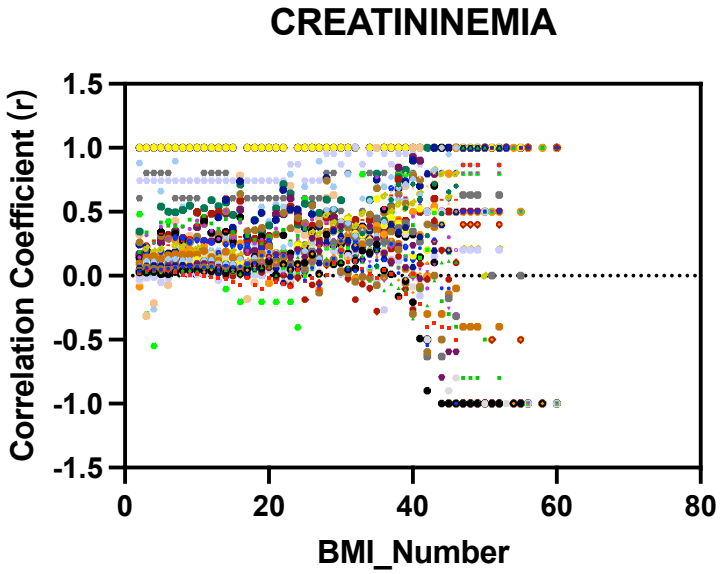


Figure 8. BMI and CREATININEMIA.
BMI is positively correlated with CREATININEMIA

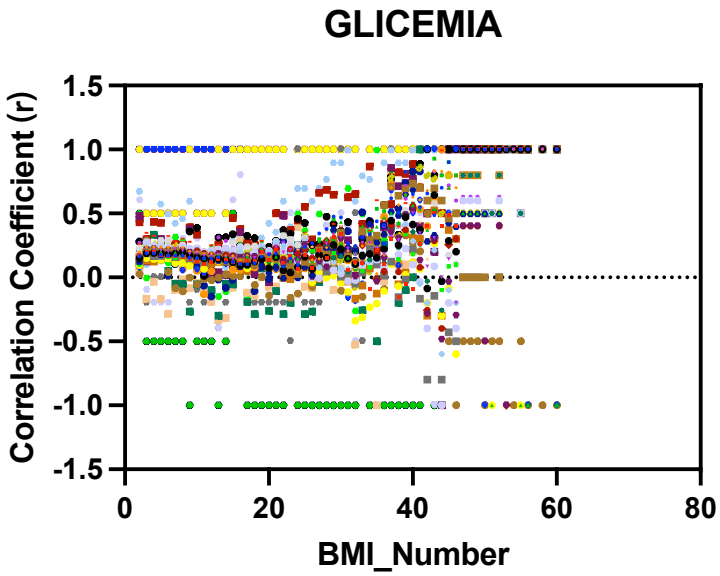


Figure 9. BMI and GLICEMIA.
BMI is positively correlated with GLICEMIA.

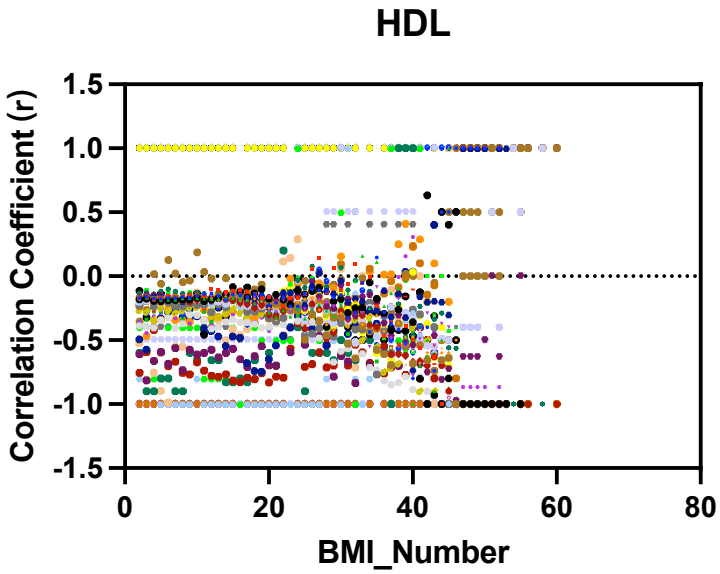


Figure 10. BMI and HDL.
BMI is negatively correlated with HDL.

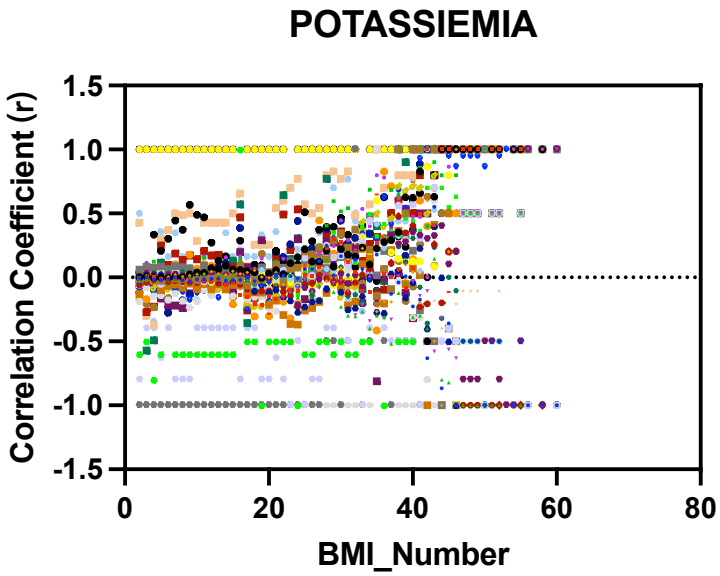


Figure 11. BMI and POTASSIEMIA.
BMI is positively correlated with POTASSIEMIA.

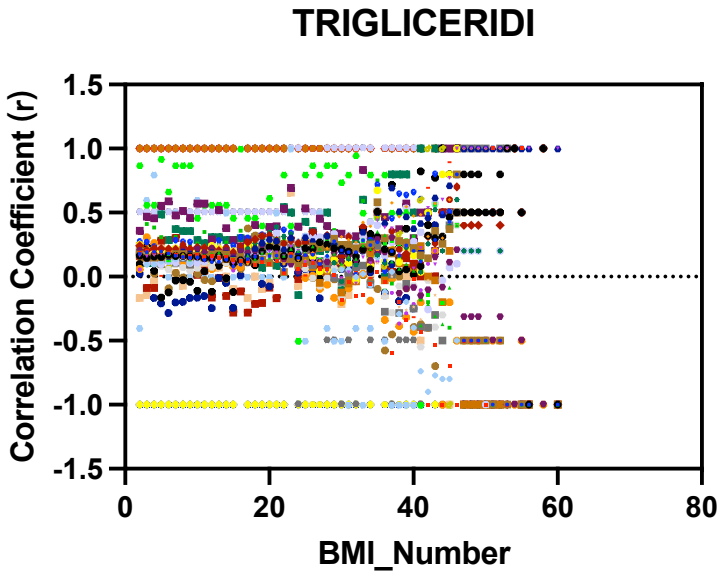


Figure 12. BMI and TRIGLICERIDI.
BMI is positively correlated with TRIGLICERIDI.

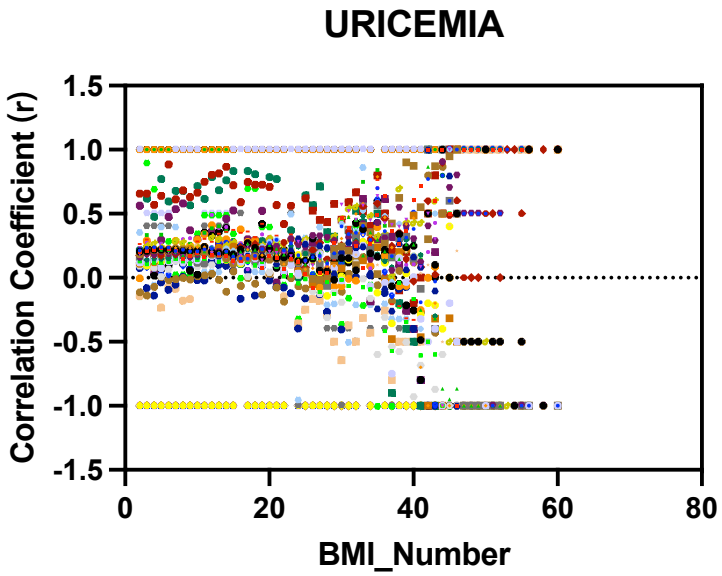


Figure 13. BMI and URICEMIA.
BMI is positively correlated with URICEMIA.

Therapies

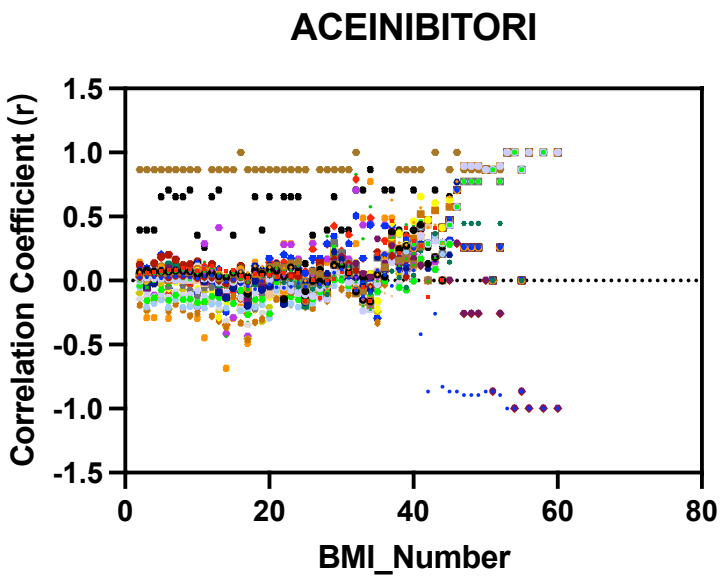


Figure 14 BMI and ACEINIBITORI.
BMI is positively correlated with the use of ACEINIBITORI.

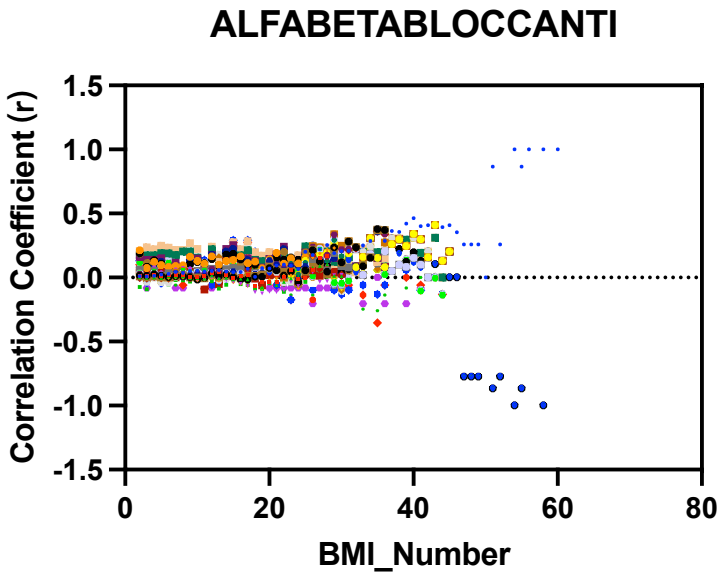


Figure 15. BMI and ALFABETABLOCCANTI.
BMI is not significantly with ALFABETABLOCCANTI (from 1 to 103).

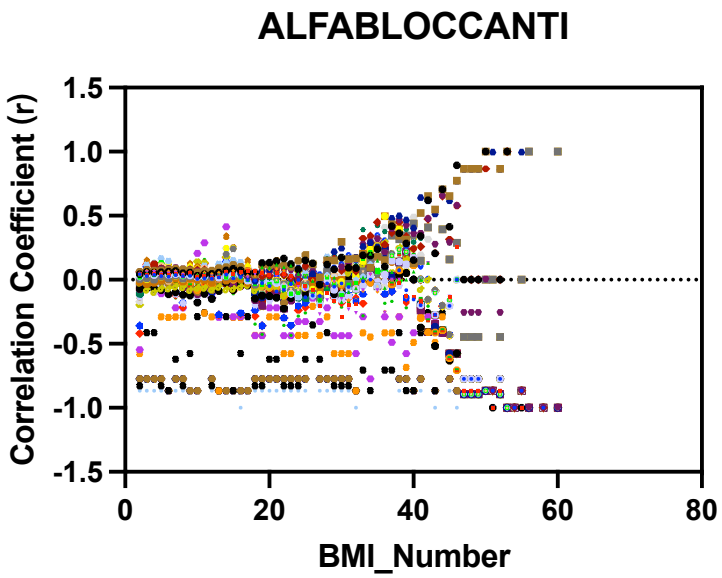


Figure 16. BMI and ALFABLOCCANTI.
BMI is not significantly with the use of ALFABLOCCANTI

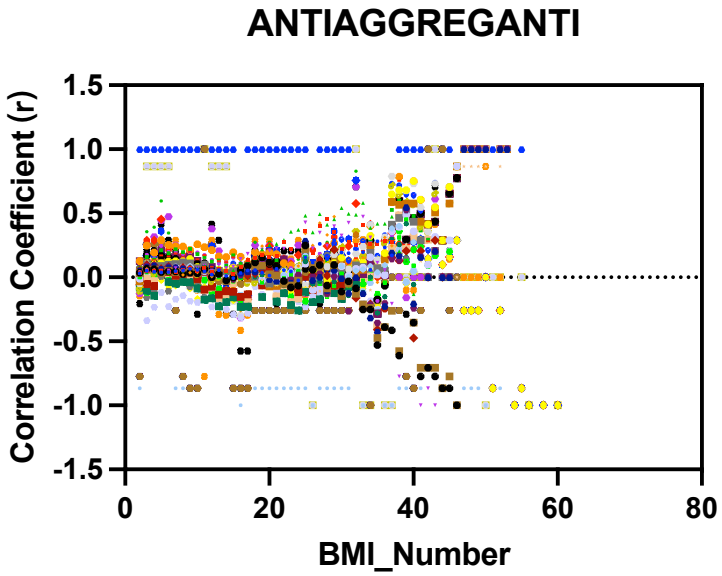


Figure 17. BMI and ANTIAGGREGANTI.
BMI is positively correlated with the use of ANTIAGGREGANTI.

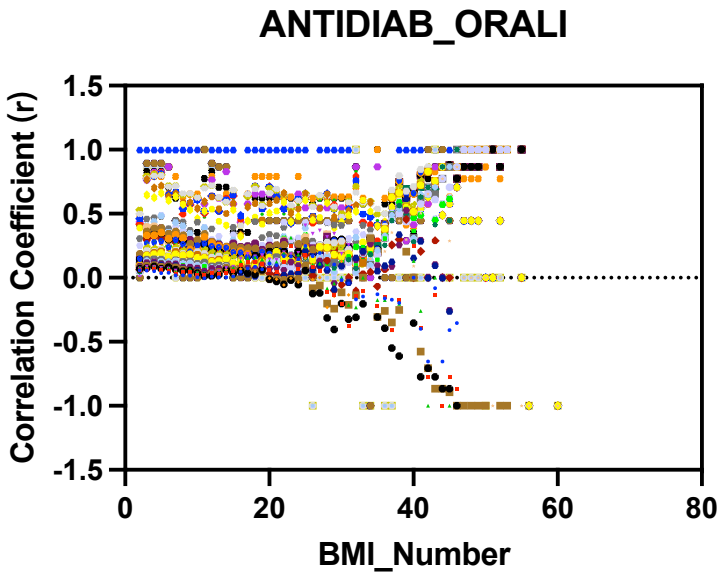


Figure 18. BMI and ANTIDIAB_ORALI.
BMI is positively correlated with ANTIDIAB_ORALI.

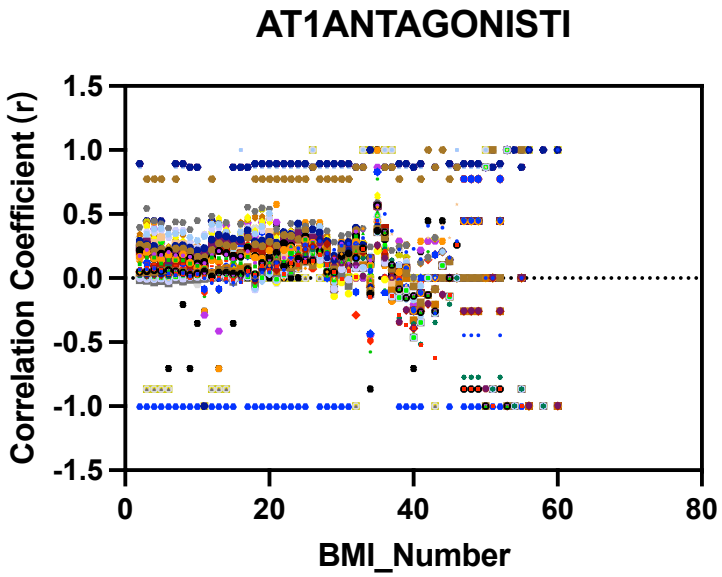


Figure 19. BMI and AT1ANTAGONISTI.
BMI is positively correlated with AT1ANTAGONISTI

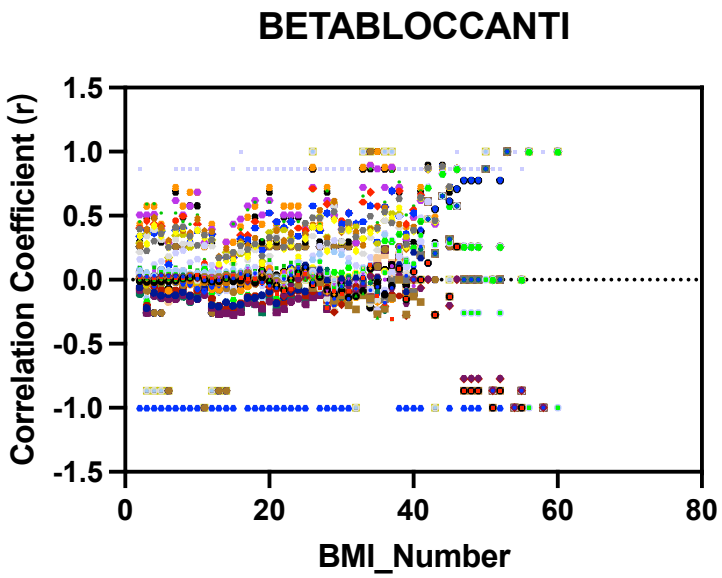


Figure 20. BMI and BETABLOCCANTI.
BMI is positively correlated with BETABLOCCANTI.

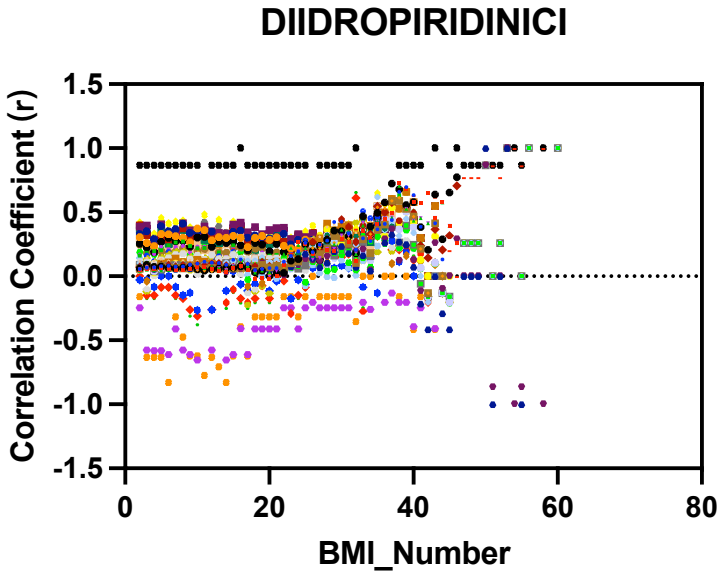


Figure 21. BMI and DIIDROPIRIDINICI.
BMI is positively correlated with DIIDROPIRIDINICI.

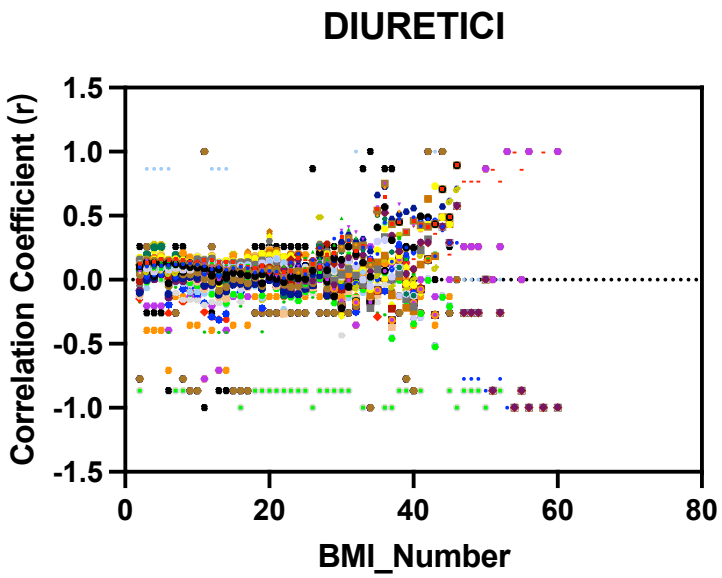


Figure 22. BMI and DIURETICI.
BMI is not significantly with DIURETICI.

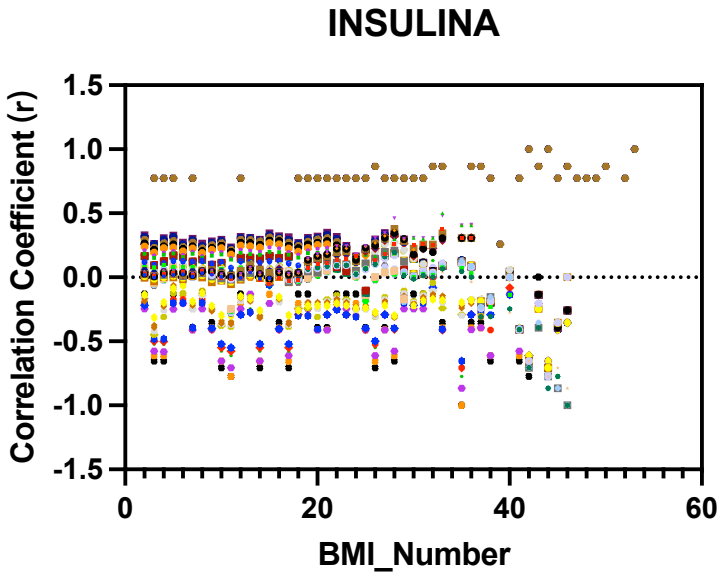


Figure 23. BMI and INSULINA.
BMI is not significantly with INSULINA.

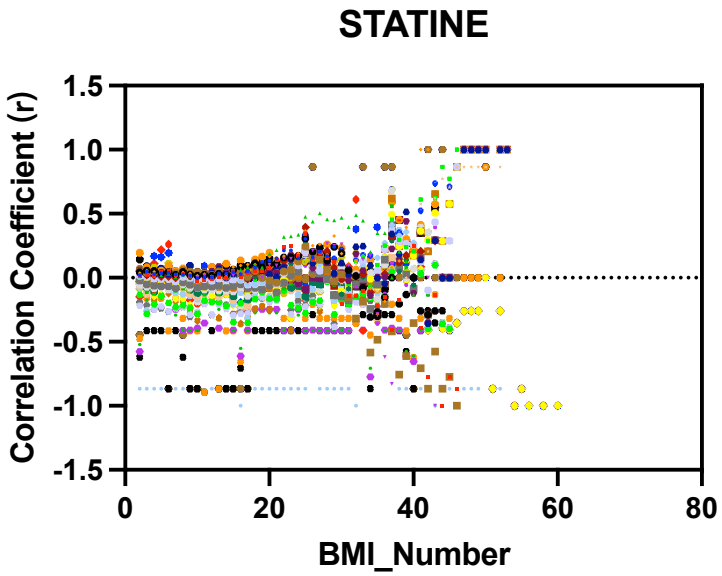


Figure 24. BMI and STATINE.
BMI is not significantly with STATINE.

Cells

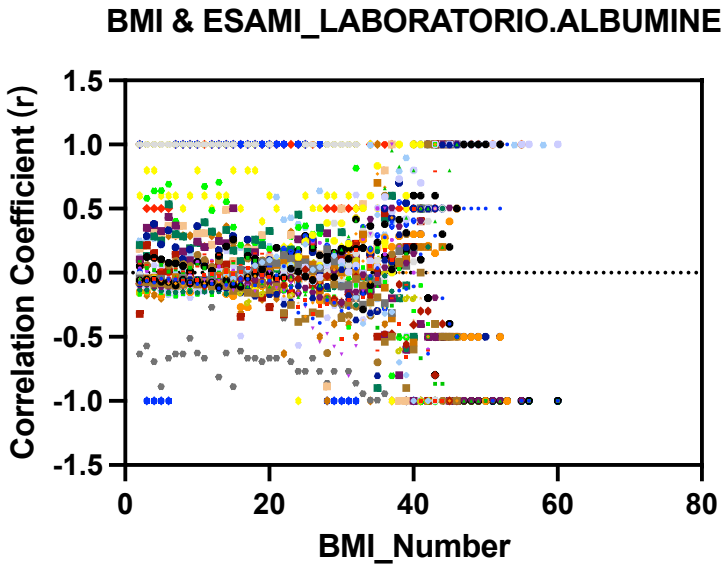


Figure 25. BMI and ESAMI_LABORATORIO.ALBUMINE.
BMI is not significantly with ESAMI_LABORATORIO.ALBUMINE.

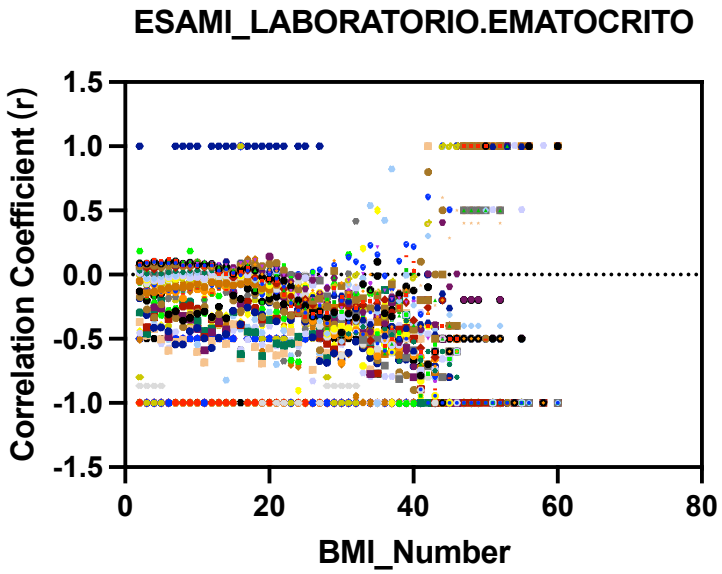


Figure 26. BMI and ESAMI_LABORATORIO.EMATOCRITO.
BMI is negatively correlated with ESAMI_LABORATORIO.EMATOCRITO.

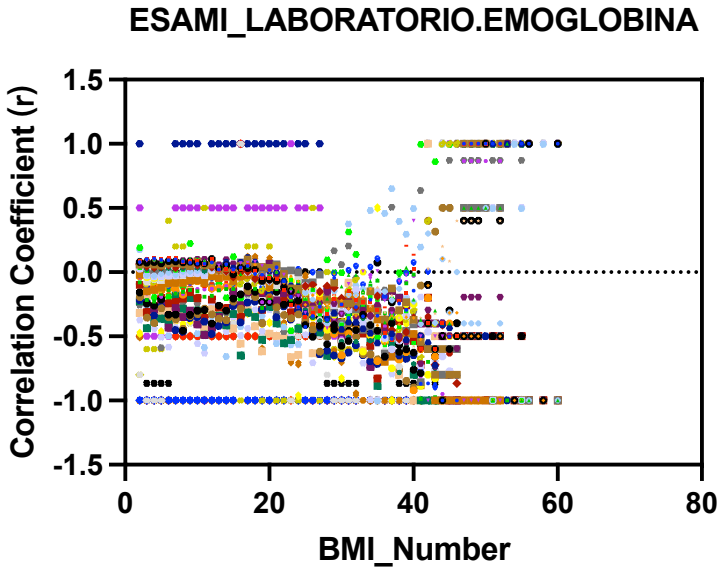


Figure 27. BMI and ESAMI_LABORATORIO.EMOGLOBINA.
BMI is negatively correlated with ESAMI_LABORATORIO.EMOGLOBINA.

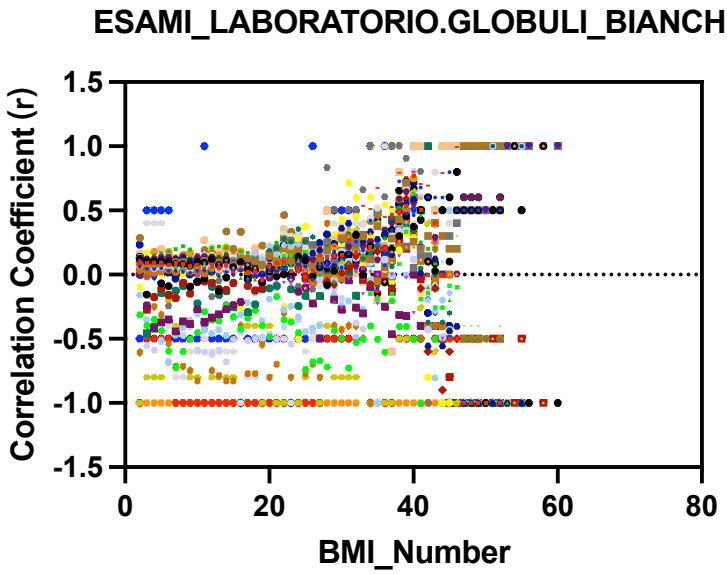


Figure 28. BMI and ESAMI_LABORATORIO.GLOBULI_BIANCHI.
BMI is not significantly with ESAMI_LABORATORIO.GLOBULI_BIANCHI.

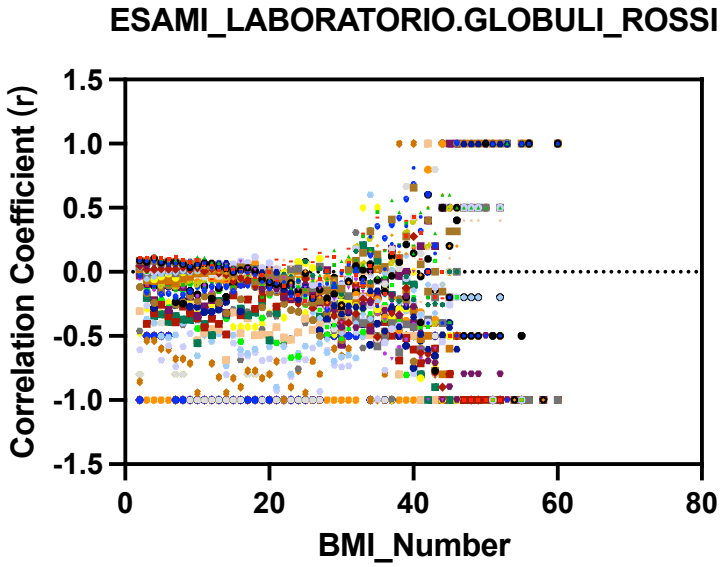


Figure 29. BMI and ESAMI_LABORATORIO.GLOBULI_ROSSI.
BMI (from 2 to 126) is positively correlated with ESAMI_LABORATORIO.GLOBULI_ROSSI.

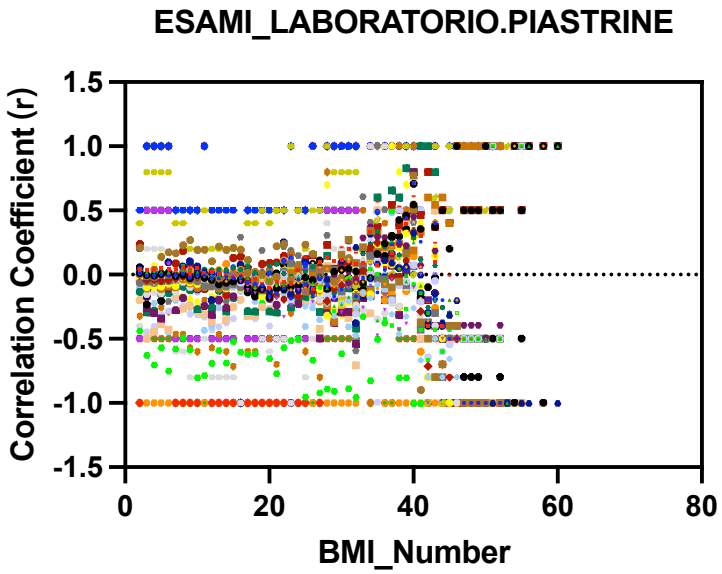


Figure 30. BMI and ESAMI_LABORATORIO.PIASTRINE.
BMI is not significantly with ESAMI_LABORATORIO.PIASTRINE.

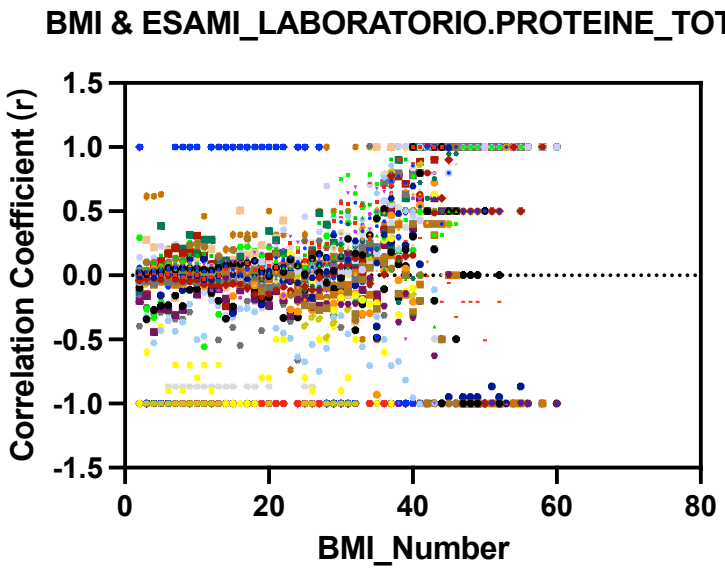


Figure 31. BMI and ESAMI_LABORATORIO.PROTEINE_TOTALI.
BMI is not significantly with ESAMI_LABORATORIO.PROTEINE_TOTALI.

Results based on delta BMI & age
Overall:
The delta BMI of (bmi_3 – bmi_2), (bmi_4 – bmi_3), (bmi_5 – bmi_4), (bmi_6 – bmi_5), (bmi_10 – bmi_9) and (bmi_13 – bmi_12) are significantly correlated with age.

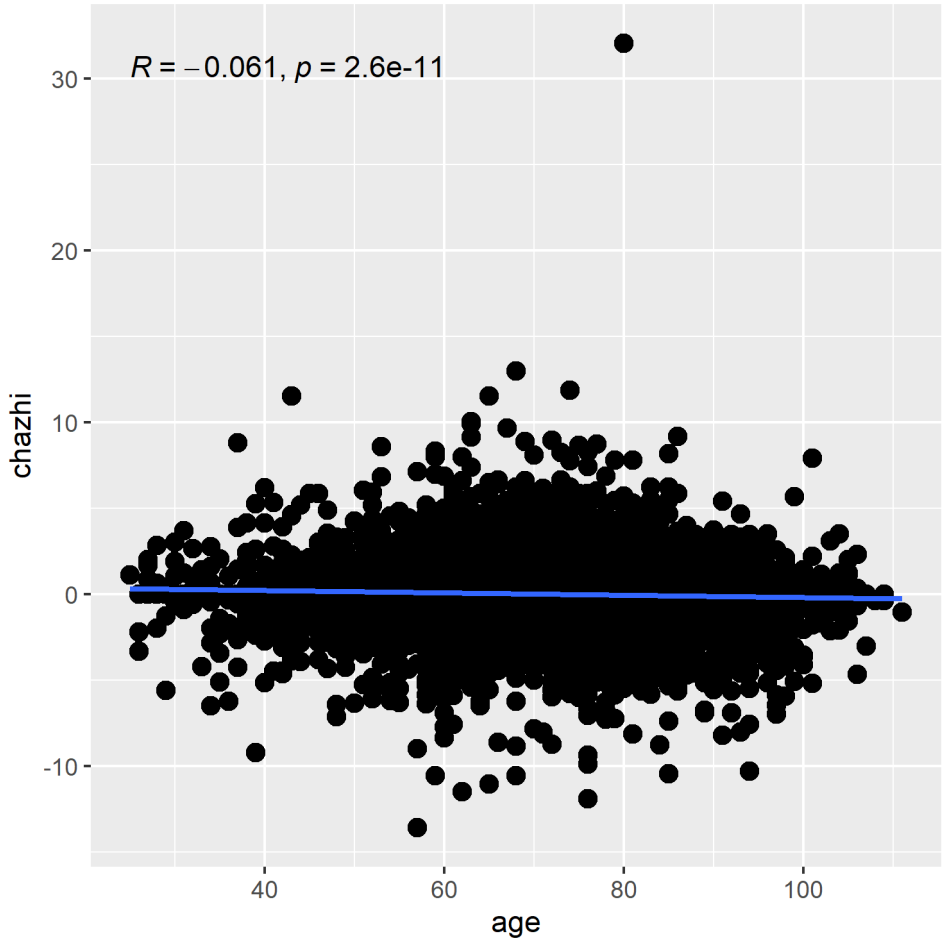


Figure 32. (bmi_3 – bmi_2) and age.
The increase from bmi_2 to bmi_3 is significantly negatively correlated with age (R=-0.061, P<0.001).

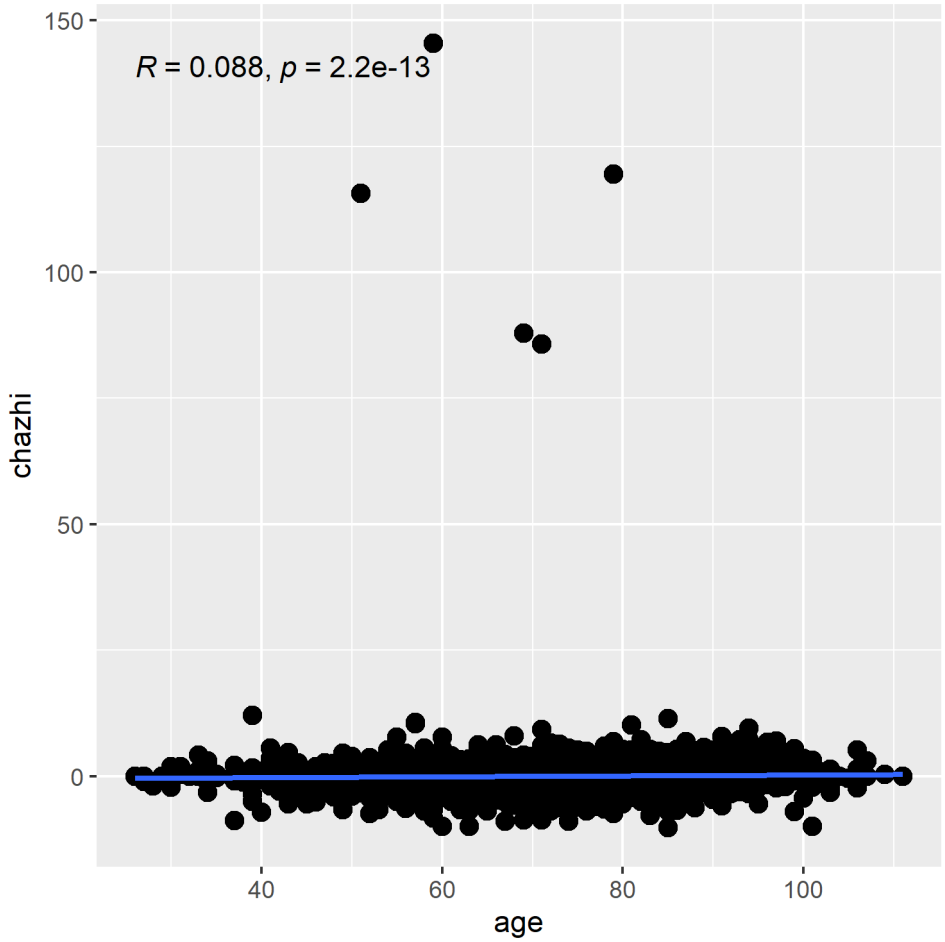


Figure 33. (bmi_4 – bmi_3) and age.
The increase from bmi_3 to bmi_4 is significantly positively correlated with age (R=0.088, P<0.001).

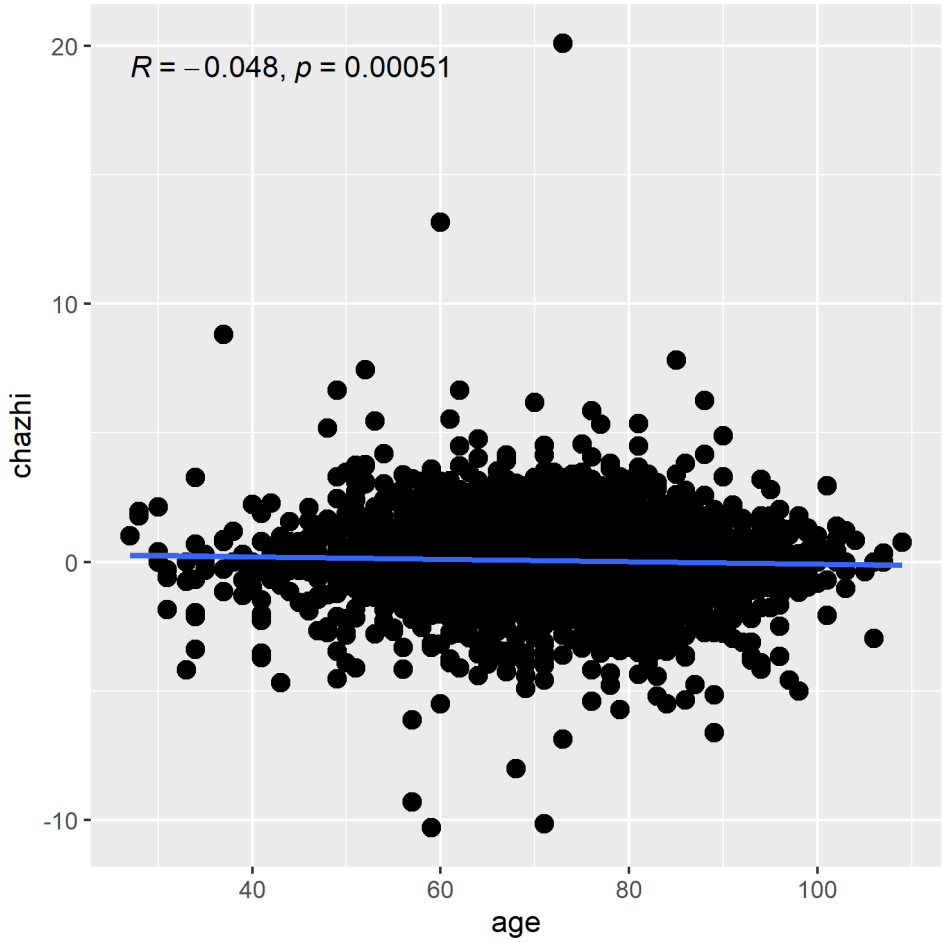


Figure 34. (bmi_5 – bmi_4) and age.
The increase from bmi_4 to bmi_5 is significantly negatively correlated with age (R=-0.048, P<0.001).

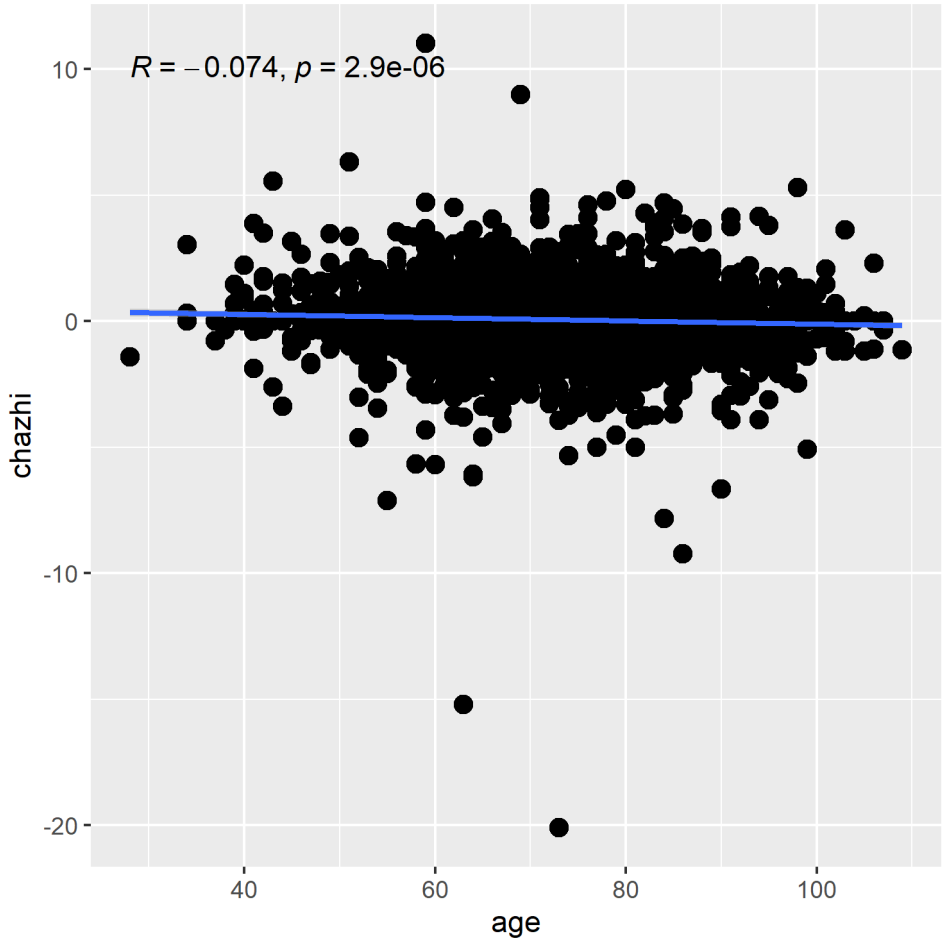


Figure 35 (bmi_6 – bmi_5) and age.
The increase from bmi_5 to bmi_6 is significantly negatively correlated with age (R=-0.074, P<0.001).

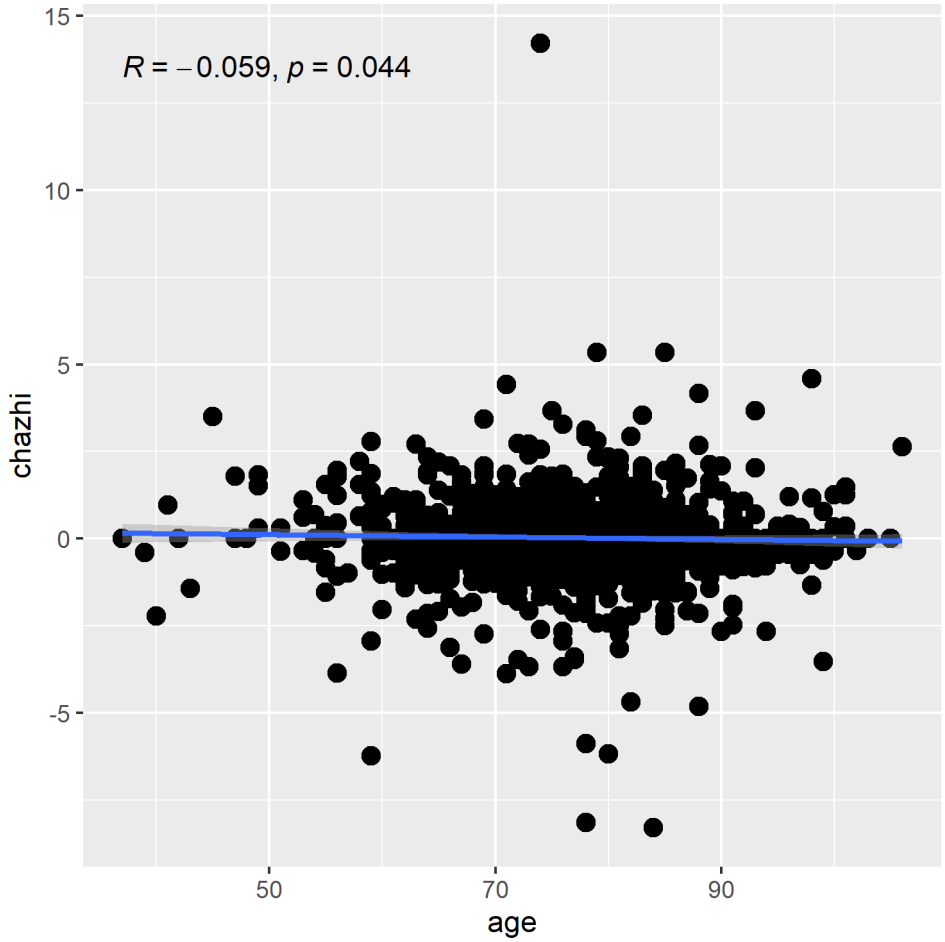


Figure 36. (bmi_10 – bmi_9) and age.
The increase from bmi_9 to bmi_10 is significantly negatively correlated with age (R=-0.059, P=0.044).

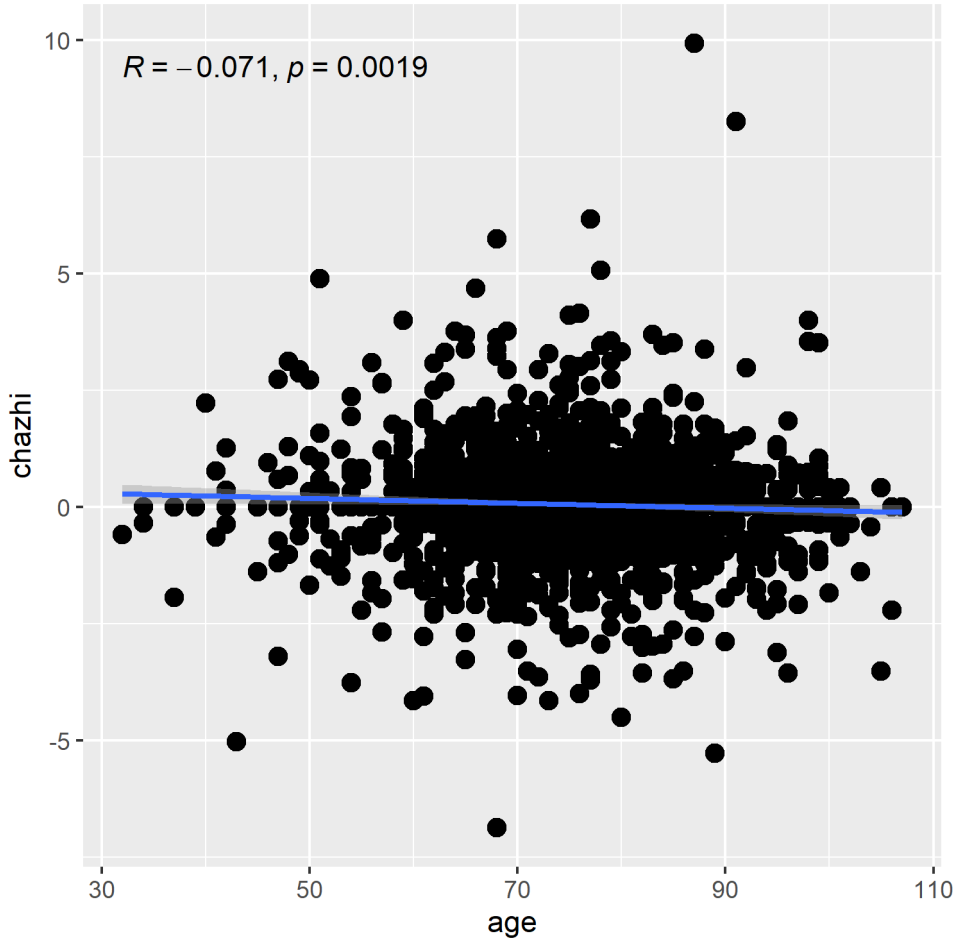


Figure 37. (bmi_13 – bmi_12) and age.
The increase from bmi_12 to bmi_13 is significantly negatively correlated with age (R=-0.071, P=0.0019).

Part III. Senocardiology and cardiongevity

Chapter 5. Senocardiology data

"Biological age as predictor for CVD disease – AI analysis of CUFII dataset"
Bischof et al. in preparation for Nature Aging, 2023.

Abstract

The risk of morbidity and mortality can be considerably decreased by early detection and care of cardiovascular diseases (CVDs), a leading cause of death worldwide. The accuracy of machine learning (ML) algorithms can be impacted by the unpredictability of blood biomarkers, which are crucial for diagnosing and prognosis of CVD. Using a sizable patient dataset with missing values and varying measurement units, we investigate the predictive capability of a DL-trained blood predictor in this work. We also look into how to more accurately predict CVD risk using additional factors, including comorbidities, medications, and ECG readings. We used a dataset of 16,336 patients, each with a distinct ID, up to 138 visits, and missing values for visit dates and biomarkers. We cleaned the dataset by grouping biomarkers according to measurement units, aligning marker sequences, removing outliers, and converting data into a base unit. After modifying the predictions using the cardio path dataset, we employed a DL-trained blood predictor, and the metrics dramatically increased. Using factors like actual age, sex, and anticipated age, we additionally examined the dataset's diagnostic and mortality statistics. We discovered that patients with diagnoses other than "Examination within the limits of the norm" had statistically older blood ages than patients with a "Normal." Additionally, we discovered that patients with older ages, sexes, and anticipated ages had greater fatality rates. Our results imply that the presumption that patients who undergo "Examination within the Limits of the Norm" are healthy may need to be reevaluated. Additional factors may enhance the precision of predicting CVD risk. In contrast to the existing biomarker-based approaches, our work suggests using ECG measures to determine CVD risk. As a result, our study emphasizes the significance of integrating additional factors to increase the precision of CVD risk prediction. According to our findings, the medical profession should reevaluate the notion that patients who have an "Examination within the Limits of the Norm" are healthy and accept the need for a more thorough approach to CVD risk prediction. The suggested use of ECG readings may offer a more precise assessment of CVD risk, enhancing patient outcomes and lowering mortality rates.

Introduction

Cardiovascular diseases (CVDs), which account for 31% of fatalities, are the main cause of death. The risk of morbidity and mortality can be decreased by early detection and therapy of CVDs. Blood biomarkers significantly influence the diagnosis and prognosis of CVD. Using blood biomarkers, machine learning (ML) algorithms can aid in predicting the risk of CVD. However, the prediction accuracy of ML algorithms can be impacted by missing values, different unit systems, and inconsistency in blood readings. Using a sizable patient dataset with missing values and varying measurement units, we evaluate the predictive capability of a DL trained blood predictor in this study. We also investigate using additional factors, such as comorbidities, medications, and electrocardiogram (ECG) readings, to predict CVD risk.

The main database, a secondary database, and metadata were all in three separate files that made up the sizable patient dataset we used. A unique ID was assigned to each of the 16,336

patients in the primary database (CODICE). The dataset had missing information for the patient's date of birth, date of visit, and up to 138 visits per patient's blood biomarkers. Using a data cleaning procedure, we could align marker sequences, cluster blood biomarkers according to measurement units, and base unitizes the data. Along with eliminating outliers, we standardized the date format. The dataset comprised 45,032 visits after cleaning, each including a maximum of 21 blood indicators. With original metrics of R2 = 0.15 and MAE = 8.7, which we modified using the cardiopath dataset, we applied a DL-trained blood predictor. The metrics were improved to R2 = 0.86 and MAE = 3.6 by modifying the predictions using the cardiopath dataset. Using factors like actual age, sex, and anticipated age, we additionally examined the dataset's diagnostic and mortality statistics.

According to our research, patients with diagnoses other than "Examination within the bounds of the norm" were likely to have older blood ages than those with a typical diagnostic. The affected set was larger than the non-afflicted group, statistically proven by the difference's modest p-value. Additionally, we discovered that patients with older ages, sexes, and anticipated ages had greater fatality rates. According to our findings, it may be necessary to reevaluate the presumption that patients who undergo "Examination within the Limits of the Norm" are healthy. The ability to predict CVD risk may be enhanced by including other factors such as the reason for the visit, medications, and comorbidities. We also suggested using ECG readings to estimate the risk of CVD.

Numerous important findings from the study conducted on the risk prediction for cardiovascular disease (CVD) are highlighted. According to the findings, patients with a diagnosis other than "Examination within the bounds of the norm" were more likely to have an older blood age than those with a normal diagnosis. This is a noteworthy finding because it raises the possibility that the idea that patients who have an "Examination within the bounds of the norm" are healthy may not be true. The medical profession must therefore reevaluate this presumption and accept the necessity for other factors, such as visit reason, medications, and comorbidities, to increase the precision of predicting CVD risk (Marioni et al., 2016).

The study also discovered that mortality rates were higher for patients with older chronological ages, higher sex ages, and higher projected ages. These findings imply that age and sex are major factors in predicting the risk of CVD. As a result, these characteristics must be considered when determining a patient's CVD risk (Mitnitski et al., 2017). The study also suggested using electrocardiogram (ECG) readings to gauge CVD risk. Given its accessibility and lack of invasiveness, ECG readings are a potentially helpful technique for estimating the risk of CVD. Healthcare practitioners could increase the accuracy of their evaluations and offer more individualized treatment regimens by including ECG measures in predicting CVD risk (Levine et al., 2013).

In conclusion, the study offers a helpful understanding of the estimation of CVD risk. The findings imply that other factors, such as the reason for the visit, medications, and comorbidities, should be considered to increase the precision of predicting CVD risk. Additionally, adding ECG measures to the CVD risk prediction may be a helpful tool for healthcare professionals. Overall, this study underscores the importance of accurately predicting CVD risk to provide patients personalized treatment plans and reduce the incidence of CVD-related mortality.

Materials and Methods

Three datasets were examined: a primary database (16336x7181), a secondary database (17761x978), and metadata. Patient data, including distinct patient IDs, names, surnames, and visit details, were stored in the main database. Blood biomarkers were included in the extra database, and the metadata described most of the primary database columns. The data's missing values, date formatting, and duplicate columns were just a few of the problems we fixed. Biomarkers were grouped into possible units using clustering algorithms, and then each cluster was converted into a base unit, and strong outliers were eliminated. In order to forecast patient age using a deep learning-based blood predictor, we next determined each patient's age at the time of the visit.

Three datasets are used in the study: a primary database with 16336 patient records, a secondary database with 17761 blood biomarker measures, and a metadata file with column descriptions. The data was cleaned and filtered using various techniques, such as aligning blood biomarker sequences, clustering biomarkers by unit, and removing too-extreme outliers. The final dataset includes 45032 unique visits, a maximum of 21 blood biomarkers, the patient's age, death date, the reason for the visit, and final diagnosis.

Initial metrics for the DL-trained blood predictor were poor, with R2 = 0.15 and MAE = 8.7. However, utilizing the cardiopath dataset to update the predictions with the average for each age, the metrics were improved to R2 = 0.86 and MAE = 3.6. According to the study, a statistically significant difference existed between the estimated blood age of the affected patients' group and the non-affected group. There were 10706 patients in the group with the diagnosis "Examination within the boundaries of the norm," compared to 10336 with other diagnoses. The study also looked at two specific features, Older and Younger, which depend on adjusted expected age, chronological age, sex, and mortality risk prediction.

According to their diagnoses, we divided the patients into two categories: those with the diagnosis "Examination within the limits of the norm" and those with a different diagnosis. Using t-tests, we compared the variation in predicted blood age and chronological age between these two groups. Using covariates like expected age, sex, chronological age, and mortality in the dataset, we also looked at these factors. We developed specific features like Older and Younger to investigate the connection between mortality and anticipated age.

Results

Our final dataset had 45032 distinct visits, a maximum of 21 blood biomarkers, the patient's age, death date, the reason for the visit, and final diagnosis. For each test, there were typically 4–9 non-missing biomarkers. R2=0.15 and MAE=8.7 were the initial measurements for the cardiopath dataset-based deep learning-based blood prediction. The metrics increased to R2=0.86 and MAE=3.6 when forecasts were adjusted using the average for each age.

Among the diagnoses in the dataset, Left Ventricular Hypertrophy and IVSin. Concentric type, CAD with EF > 50%, and IVSin. The eccentric type was the most common. Our research revealed that patients with diagnoses other than "Examination within the limits of the norm" had older projected blood ages than patients with normal diagnoses (p-value 0.05). We also discovered that age, sex, and anticipated age were significant predictors of death (p-value 0.05).

The study discovered a statistically significant difference in projected blood age between the patients diagnosed with "Examination within the boundaries of the norm" and those with other diagnoses. The affected patients' group was anticipated to be older than the unaffected group. Because of the statistically significant discrepancy, the study rejected the null hypothesis that the averages were equal. With the help of the unique features of Older and Younger, the mortality risk prediction model that considered covariates, including chronological age, sex, and anticipated blood age, produced encouraging findings.

Original data stats

Data consists of three files:

1. Main database 16336x7181
    a. Unique ID column name
    b. Each row is an unique patient according to CODICE column (but Name+Surname columns contain 13325 unique records)
    c. Maximum amount of visits are 138
2. Additional database 17761x978
    a. Additional blood biomarkers
    b. Unique ID column name
    c. There are maximum of 21 biomarkers present both in the database+additional database and in DL blood predictor
3. Meta data
    a. Descriptions for most of the columns from main database

Data stats after cleaning

Filtering steps were conducted:

1. Find all existing columns for each blood marker and define align for each marker sequence in order to concatenate them and correlate with exact visit
2. Keep visits with existing date of birth and date of visit
3. Cluster each marker according to possible units. Then, transform each cluster to a base unit. Drop all strong outliers
4. Transform all dates in the same format and calculate age at the time of the visit

Final datasets consist of 45032 unique visits with a maximum of 21 blood biomarkers, age at the time of the visit, date of death, cause of visit and final diagnosis.

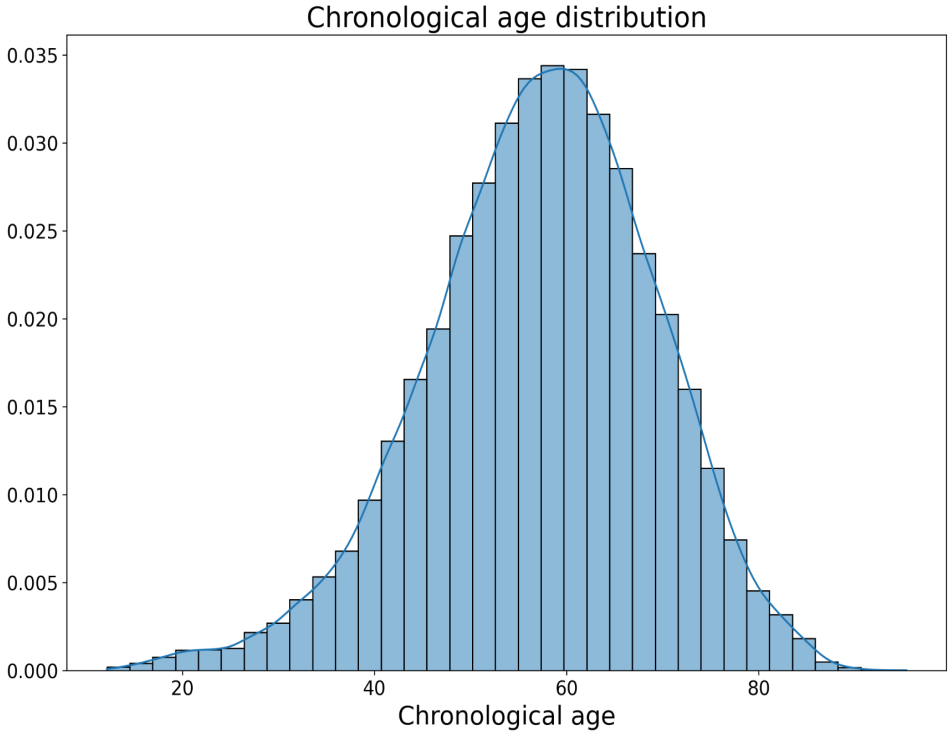


Figure 1. Chronological age distribution

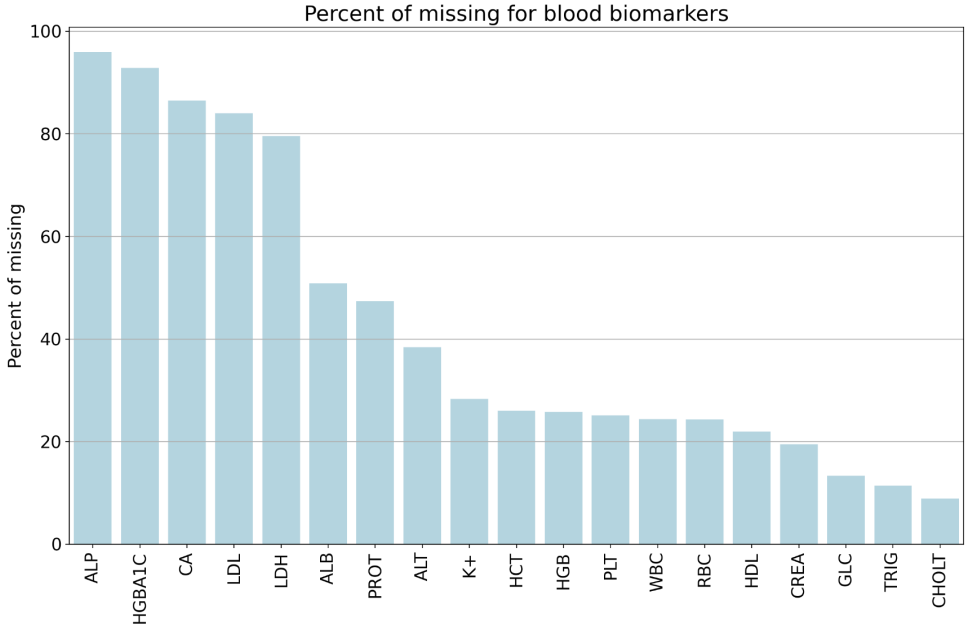


Figure 2. Percent of missing for blood biomarkers

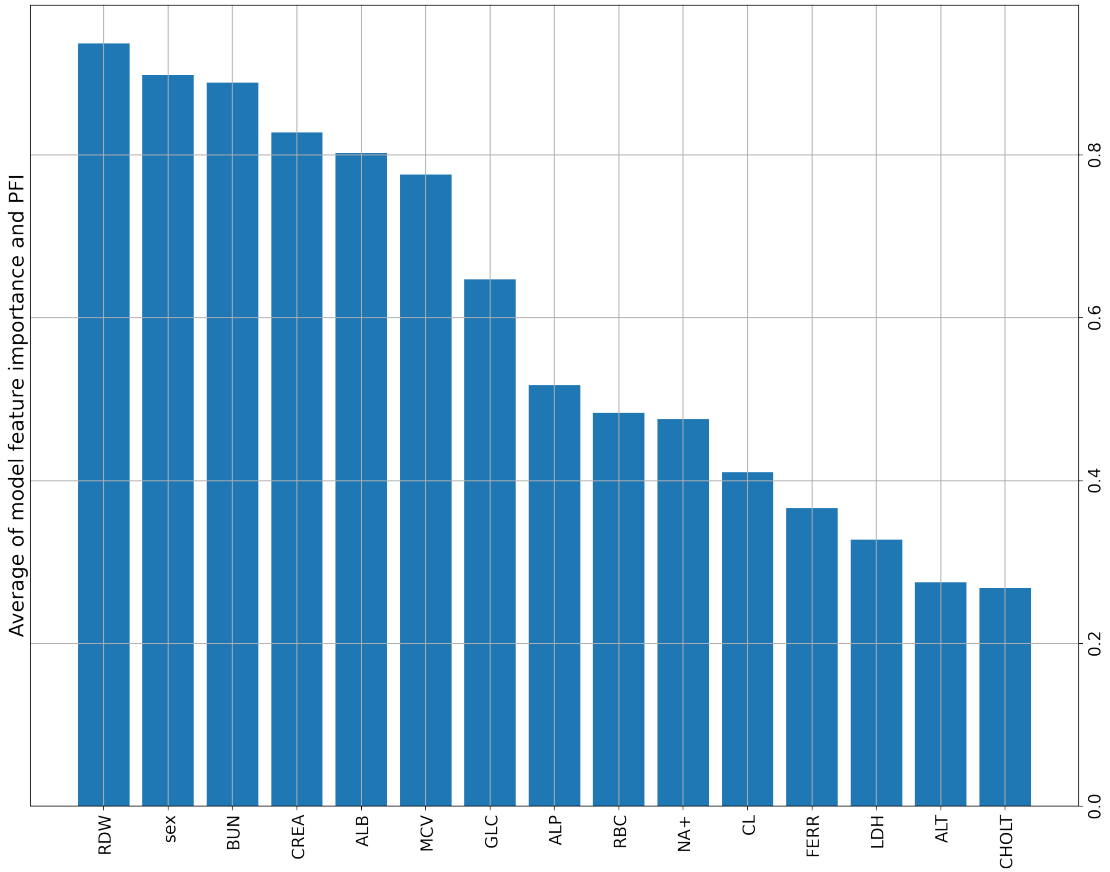


Figure 3. Average of model feature importance and PFI
There are 4-9 non-missing biomarkers for each test by average

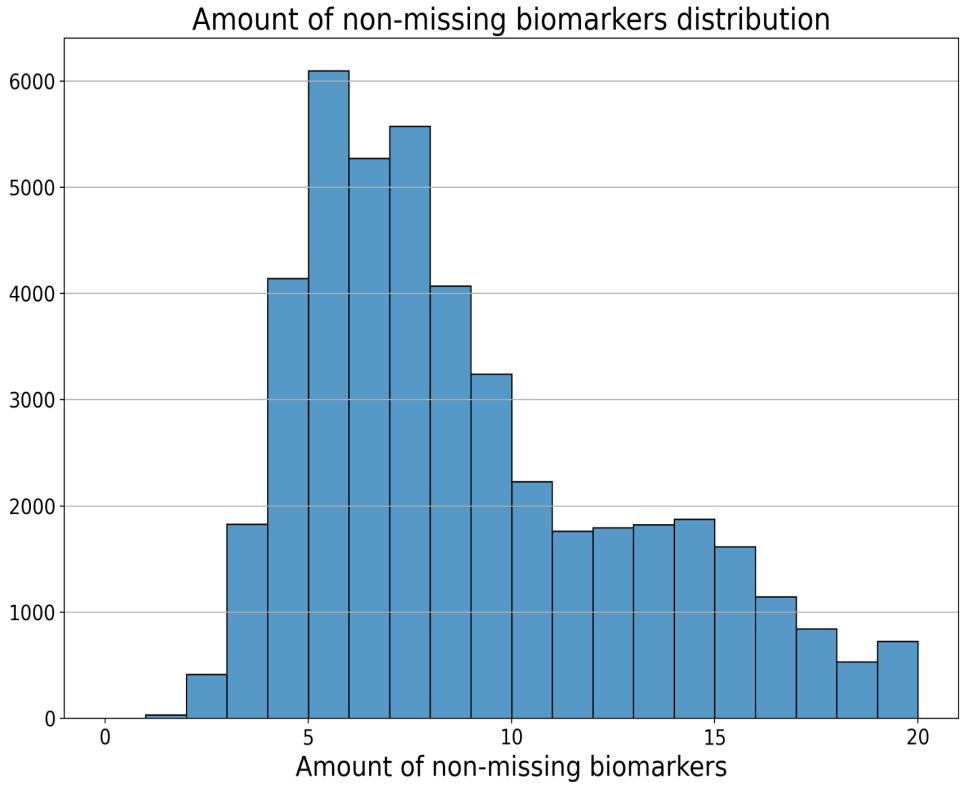


Figure 4. Amount of non-missing biomarkers distribution

Predictor metrics & adjusting

DL trained blood predictor original metrics using cardiopath dataset are R2 = 0.15; MAE = 8.7

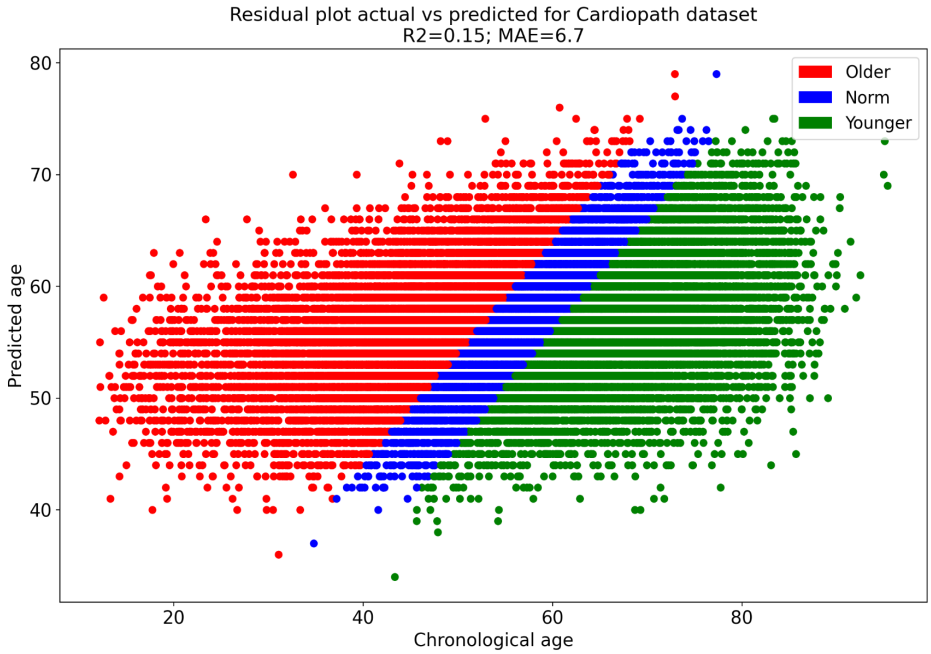


Figure 5. Residual plot actual & predicted for Cardiopath dataset

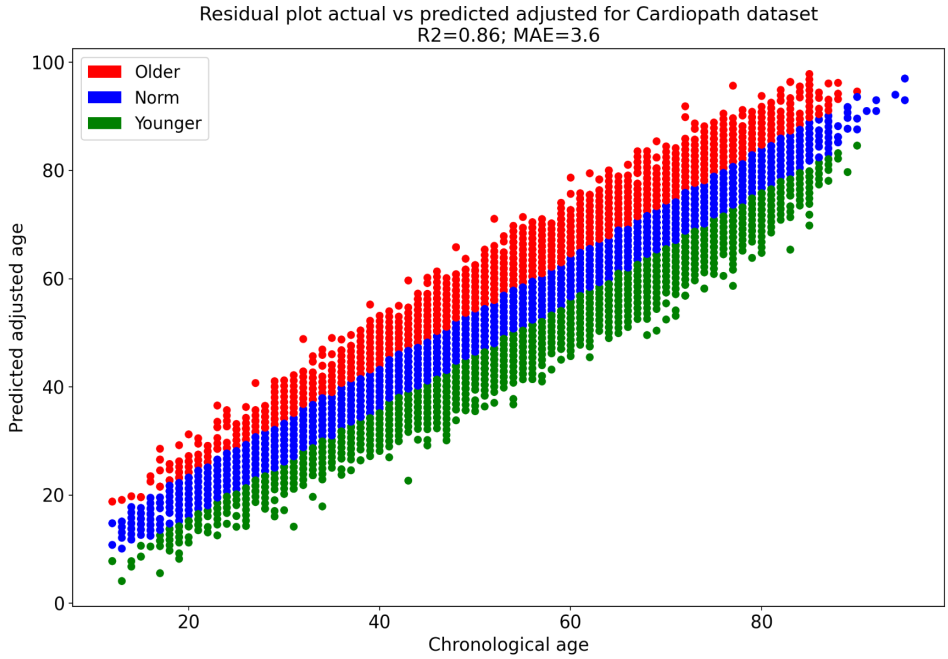


Figure 6. Residual plot actual & predicted adjusted for Cardiopath dataset

Metrics are not high first of all because of small average amount of non-missing biomarkers. But using all cardiopath dataset we can adjust predictions with average for each separate age. Using this technique our metrics become R2=0.86 and MAE = 3.6

Diagnosis stats

We would assume that afflicted patients have a diagnosis different from “Examination within the limits of the norm”. There are ~1200 unique diagnoses, the most frequent are:

Table 1. Amount of different Diagnosis

Diagnosis (It)	**Diagnosis (Eng)**	**Amount**
Ipertrofia ventricolare sinistra	Left ventricular hypertrophy	6885
IVSin. di tipo Concentrico	IVSin. Concentric type	305
CAD con EF > 50 %	CAD with EF> 50%	253
IVSin. di tipo Eccentrico	IVSin. Eccentric type	234
Cardiopatia valvolare aortica	Aortic valvular heart disease	125
Cardiopatia valvolare mitralica	Mitral valve heart disease	93
CAD con EF< 50 %	CAD with EF < 50%	62

A group of patients with diagnosis “Examination within the limits of the norm” (10706 patients) vs a group of patients with another diagnosis (everything which is not normal) diagnosis (10336 patients). For both groups blood age was predicted and deltas between blood age and chronological age were compared.
Additionally, we provide statistical difference between two groups deltas using t-test p-value

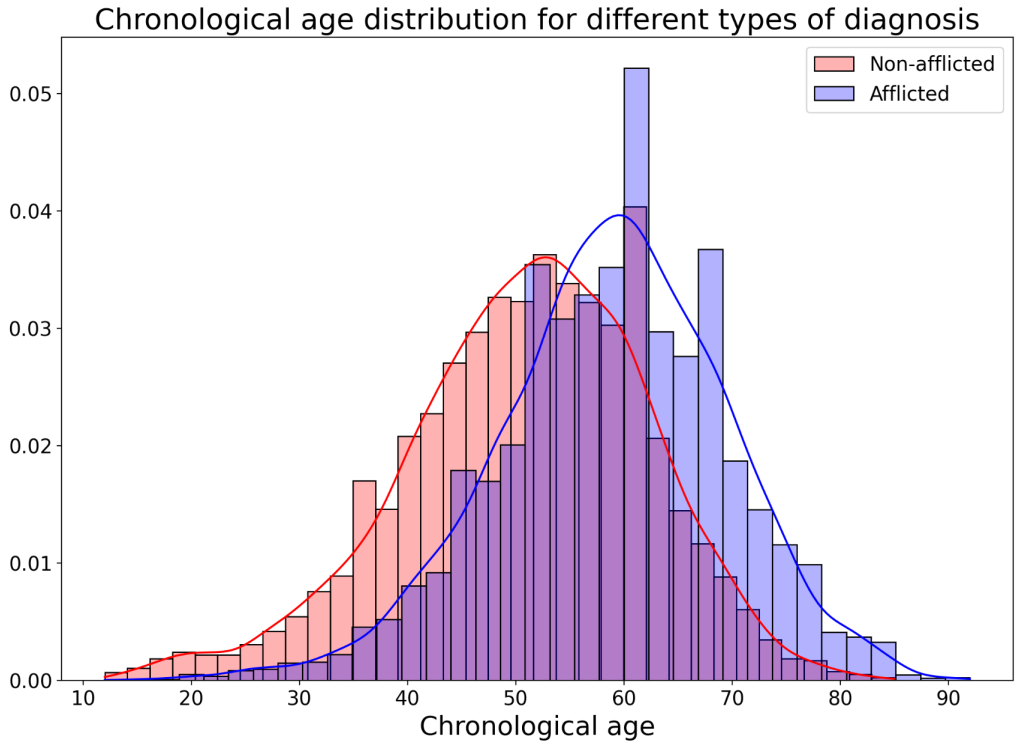


Figure 7. Chronological age distribution for different types of diagnosis
Age distributions are not balanced, but we use adjusted predictions and deltas, so this dependence is normalized by chronological age

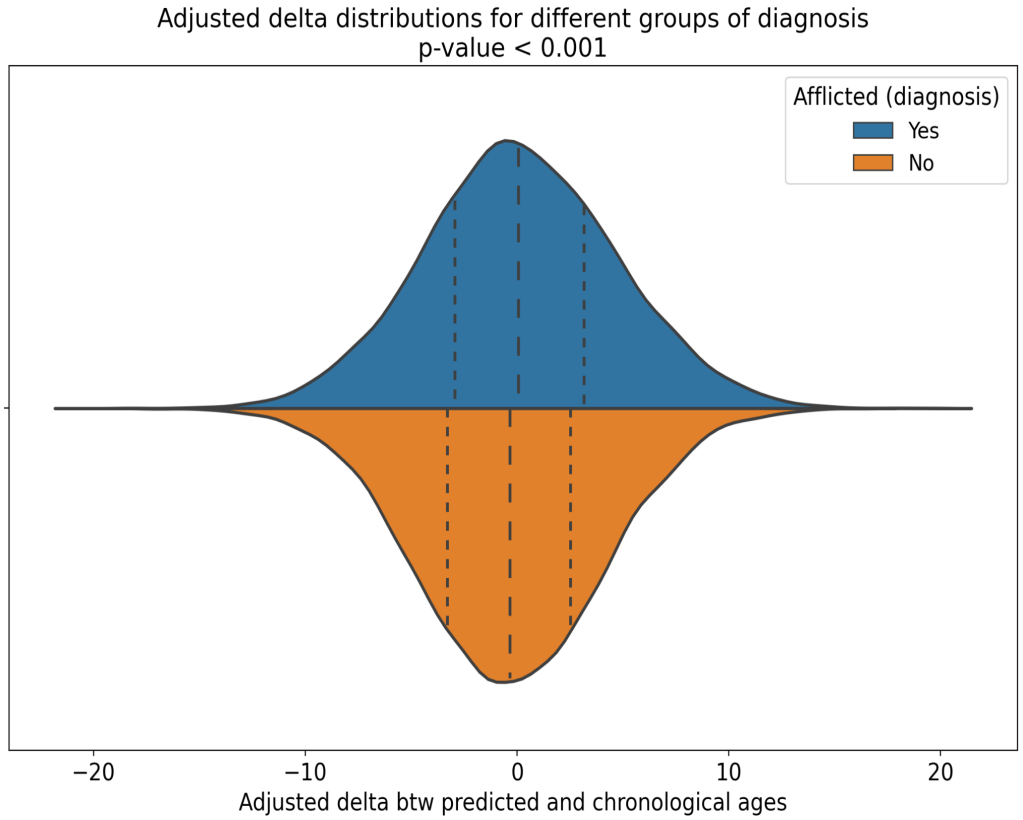


Figure 8. Adjusted delta distribution for different groups of diagnosis

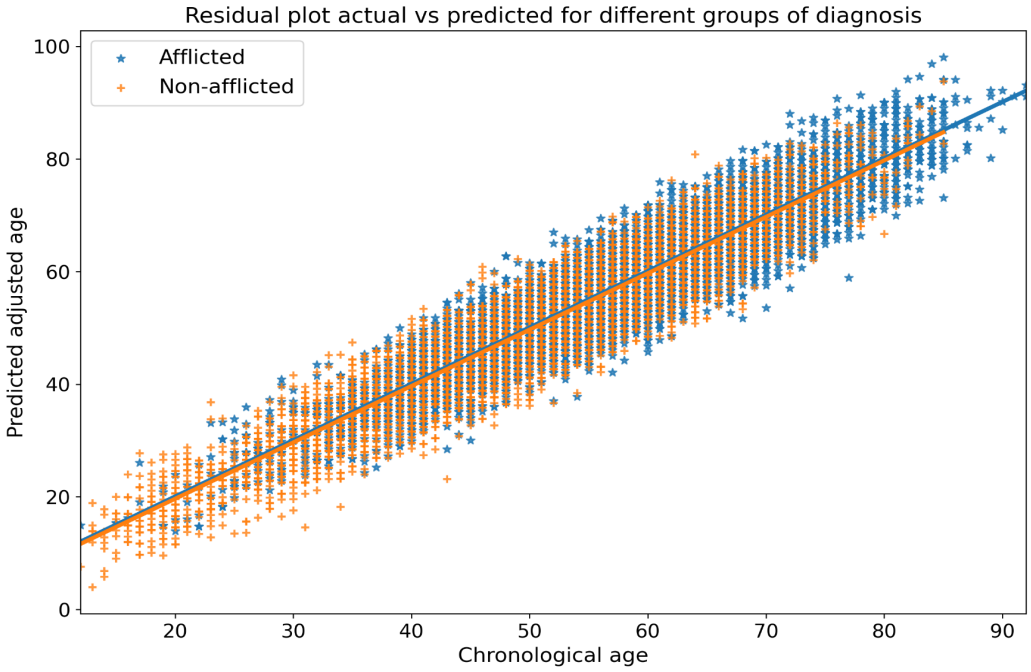


Figure 9. Residual plot actual & predicted for different groups of diagnosis

A group of afflicted patients are predicted older than other patients (the difference is statistically significant). The difference is statistically significant, we reject the null hypothesis of equal averages because of small p-value and cannot reject the alternative hypothesis, that the afflicted set is greater than non-afflicted one.

Mortality

Only 284 samples from the data have a date of death. We would assume that everyone else did not die.
Covariates used for each mortality test are chronological age, sex, and two special features **Older** and **Younger** which depends on adjusted predicted age:

- Older = 1, if predicted_age - age >= Delta; Older = 0 otherwise
- Younger = 1, if predicted_age - age >= Delta; Younger = 0 otherwise

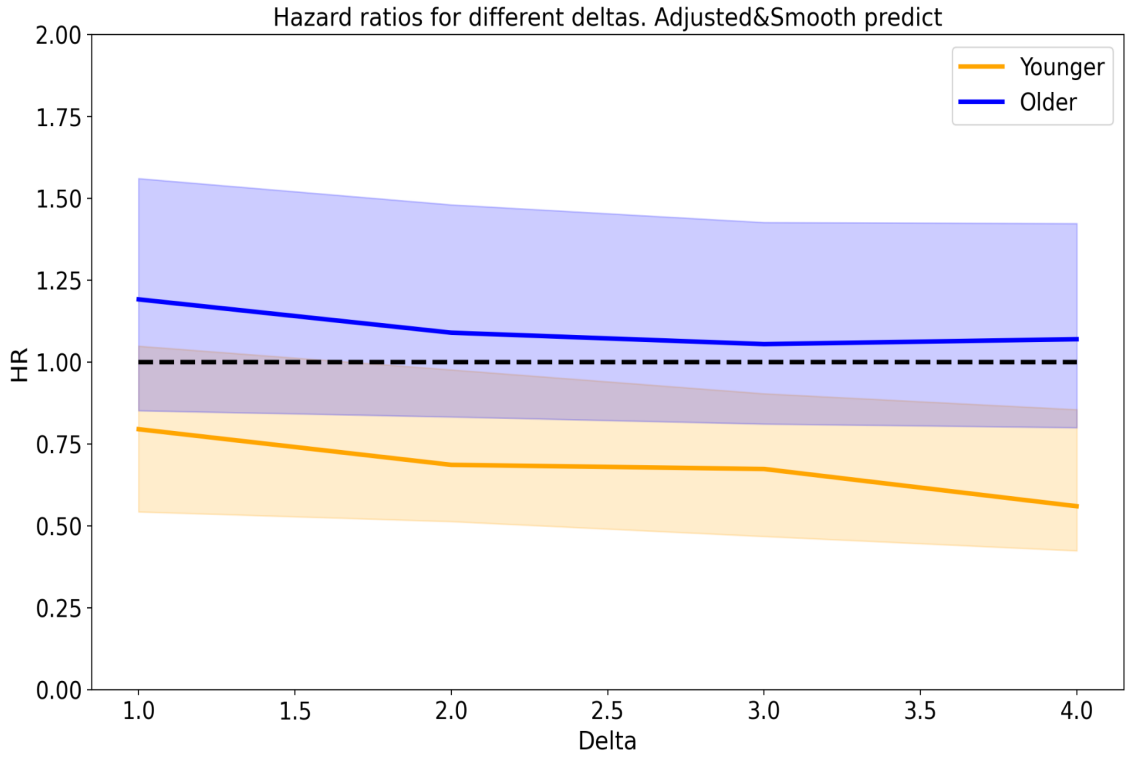


Figure 10 Hazard ratios for different deltas. Adjusted & Smooth predict**.**

Delta	Older. p-value	Younger. p-value	Older. Not dead	Older. Dead	Younger. Not dead	Younger. Dead
1	0.28	0.19	18107.0	138.0	18338.0	94.0
2	0.53	0.02	14161.0	107.0	14496.0	70.0
3	0.70	0.01	10591.0	77.0	11060.0	53.0
4	0.65	0.01	7627.0	57.0	8054.0	32.0

Table 2. Survival situation of different deltas

Discussion

This study's results show ML algorithms' capability to forecast CVD risk using blood biomarkers. However, the prediction accuracy of ML algorithms can be impacted by missing values and variations in measurement units. Our data-cleaning procedure increased the blood predictor's accuracy from R2 = 0.15 and MAE = 8.7 to R2 = 0.86 and MAE = 3.6. Our analysis also demonstrated that adding new factors, such as the reason for the visit, medications, and comorbidities, may increase the precision with which CVD risk can be

predicted. ECG readings can also be used to predict CVD risk as another covariate. The findings of this study point to the requirement for a patient dataset that is more complete and contains additional variables and ECG readings.

According to the study's findings, clinical diagnosis in a cohort of patients is correlated with anticipated blood age. Comparing the affected group of patients to the unaffected group, there was a statistically significant difference in projected blood age. These results imply that anticipated blood age might be a valuable diagnostic for locating people at high risk for CVD. It may also be helpful to identify patients who are at high risk of dying using the mortality risk prediction model that takes chronological age, sex, and anticipated blood age into account. But other factors, such as the reason for the visit, medications, and comorbidities, might increase the model's accuracy. The analysis of electrocardiogram (ECG) data could also benefit the study. The mortality risk prediction model might benefit from additional data that comes from age prediction using 1-lead ECG records.

The study's assumption about "Examination within the limits of the norm" patients as healthy patients should be further investigated. These patients may have a low risk of CVD but still have other health risks that should be considered. The study's assumption about alive/dead patients also needs further investigation, as only 284 samples had a date of death. Using ML algorithms to predict CVD risk using blood biomarkers is an active research area. The study by Vanitha et al. (2022) has demonstrated the potential of DL algorithms in this regard. However, as highlighted in the study, missing values and differences in measurement units can affect the accuracy of the predictions made by ML algorithms. The data cleaning process used in this study effectively improved the accuracy of the DL-trained blood predictor, which is an important step towards making more accurate CVD risk predictions.

According to the Soriano-Tárraga et al. (2016) study, ischemic stroke patients are physiologically older than physically. This conclusion is important for predicting CVD risk because age is a significant risk factor. Additionally, Tanaka et al. (2018) discovered that healthy adults had a plasma proteome signature of aging that may help predict the risk of CVD. According to studies by Weidner et al. (2014), Zbie-Piekarska et al. (2015), and Zhang et al. (2019), DNA methylation alterations have also been identified as potential aging markers. Additionally, it has been demonstrated that DNA methylation patterns in peripheral blood can accurately predict all-cause mortality (Zhang et al., 2017), highlighting the possibility of employing epigenetic markers in CVD risk prediction.

In addition to blood biomarkers and age, other covariates such as cause of visit, drugs, and comorbidities were found to potentially improve the prediction accuracy of CVD risk in the study by Vanitha et al. (2022). ECG measurements were also a potential covariate in CVD risk prediction. The study by Xia et al. (2019) also highlights the potential of using multi-modality networks for CVD risk prediction, including imaging and clinical information. The development of a clinical decision support system for severity risk prediction and triage of COVID-19 patients at hospital admission has also been reported (Wu et al., 2020). This highlights the potential of using ML algorithms in healthcare to improve patient outcomes.

Predicting conditions like dysglycemia, hypertension, and heart illnesses has become increasingly common thanks to machine learning algorithms. One study by Absar et al. (2022) showed the effectiveness of smart systems aided by machine learning in predicting cardiac disease. Similarly, AlKaabi et al. (2020) predicted hypertension using machine learning, and Ali et al. (2019) created a prediction model for heart disease using these same techniques. In

order to detect dysglycemia from an ambulatory ECG, Chiu et al. study .'s from 2022 also used tailored machine learning.

Furthermore, using machine learning algorithms in medical image analysis has gained attention. For instance, Dharwadkar and Savvashe (2021) used a modified convolutional neural network to segment right ventricle MRI images. Additionally, Attallah and Ragab (2023) developed an automatic diagnosis of myocardial infarction using multiple GLCMs, CNNs, and SVMs.

Apart from predicting diseases, machine learning algorithms can also predict biological age. Bize et al. (2009) showed that telomere dynamics, rather than age, predict life expectancy in the wild. Moreover, Bocklandt et al. (2011) developed an epigenetic predictor of age, while Borkan and Norris (1980) assessed biological age using a profile of physical parameters. Breitling et al. (2016) found that frailty is associated with the epigenetic clock but not telomere length. This work illustrates the potential of machine learning (ML) algorithms for risk prediction of cardiovascular disease (CVD) using blood biomarkers. The results imply that cleaning the data can enhance the ML algorithms' accuracy, which is important when dealing with missing values and variations in measurement units. Additionally, more factors can increase the accuracy of predicting CVD risks, such as visit reason, medications, and comorbidities. Electrocardiogram (ECG) readings can also be utilized as another covariate to forecast the risk of CVD. These results support a prior study that showed the value of ML in predicting the risk of CVD (Gowri et al., 2022; Kim et al., 2022; Louridi et al., 2021).

The study's results also highlight the significance of having a thorough patient dataset that includes extra variables and ECG readings to enhance CVD risk prediction accuracy. Additionally, numerous studies have emphasized the value of biological age predictors, such as DNA methylation and epigenetic aging markers, in anticipating age-related illnesses (Hannum et al., 2013; Horvath et al., 2015; Jones et al., 2015; Koch & Wagner, 2011; Levine et al., 2018). The results of this study contribute to the expanding body of literature that has shown the potential of ML algorithms in predicting CVD risk and the significance of a thorough patient dataset that contains extra variables and ECG readings to increase prediction accuracy.

Absar et al. (2022) found that machine-learning-supported smart systems can accurately predict heart disease. Similarly, AlKaabi et al. (2020) demonstrated the effectiveness of machine learning in predicting hypertension. Attallah and Ragab (2023) also used machine learning to diagnose myocardial infarction. Chiu et al. (2022) utilized personalized machine learning to screen for dysglycemia from ambulatory ECG towards non-invasive blood glucose monitoring. Besides predicting and diagnosing medical conditions, machine learning can also estimate biological age. For instance, Bize et al. (2009) found that telomere dynamics better predict life expectancy than chronological age in the wild. Björkman et al. (2012) demonstrated that the pharmacokinetics of recombinant factor VIII are related to age and body weight. Bocklandt et al. (2011) developed an epigenetic predictor of age. Breitling et al. (2016) found that frailty is associated with the epigenetic clock but not telomere length. Fiorito et al. (2017) studied the association between social adversity and epigenetic aging.

Machine learning can also be used to predict treatment outcomes. Engmann et al. (1999) demonstrated the value of ovarian stromal blood flow velocity measurement in predicting ovarian responsiveness and the outcome of in vitro fertilization treatment. De Lange et al. (2020) utilized multimodal brain-age prediction to assess cardiovascular risk. Dharwadkar

and Savvashe (2021) developed a modified convolutional neural network for the right ventricle segmentation of magnetic resonance images. Nagrajan et al. (2016) used machine learning techniques to create a powerful prediction model for cardiac disease. The Cleveland Heart Disease dataset was used, which includes non-invasive and clinical variables for heart disease prediction. The authors compared the performance of numerous machine learning algorithms and discovered that the decision tree approach performed better than the others. The study offers insightful information regarding the efficiency of the decision tree algorithm for heart disease prediction.

Similarly to this, data mining approaches were employed by Rairikar et al. (2017) to forecast cardiac disease. Several data mining algorithms, including decision trees, K-NN, and Naive Bayes, were used on the same dataset for Cleveland Heart Disease. The authors discovered the decision tree algorithm to be the most effective technique for heart disease prediction. Pan et al. (2020) proposed an enhanced deep learning-assisted convolutional neural network (CNN) for heart disease prediction on the Internet of Medical Things (IoMT) platform. The study utilized the Pima Indians Diabetes dataset, which includes features such as age, blood pressure, and body mass index. The authors found that their proposed CNN model outperformed another accuracy, sensitivity, and specificity methods.

Palechor et al. (2017) used supervised and unsupervised data mining techniques to analyze cardiovascular disease. The authors used the Framingham Heart Study dataset and applied various data mining techniques, including decision trees, K-means, and hierarchical clustering. The study provides valuable insights into the effectiveness of these techniques for identifying risk factors associated with cardiovascular disease. Epigenetic age acceleration is also linked to cardiovascular disease (Perna et al., 2016). Peters et al. (2015) explored the transcriptional landscape of age in human peripheral blood. They found that age-related changes in gene expression can be used to predict the risk of age-related diseases, including cardiovascular disease.

Machine learning algorithms can be used to identify and categorize heart rhythm problems and predict cardiac disease (Ramani et al., 2020). K-NN and decision trees were just two of the machine learning methods used in the study, which used the MIT-BIH Arrhythmia database. The proposed decision tree algorithm by the authors was successful in identifying and categorizing arrhythmia abnormalities, according to the authors. The use of deep neural networks to create deep indicators of human aging was also investigated by Putin et al. (2016). Deep neural networks were discovered to have a high degree of accuracy in predicting cardiovascular illness and aging and age-related diseases. The study sheds important light on the potential of deep learning algorithms to find biomarkers linked to cardiovascular disease.

In conclusion, the study by Vanitha et al. (2022) demonstrates the potential of using ML algorithms to predict CVD risk using blood biomarkers. However, the accuracy of these predictions can be affected by missing values and differences in measurement units. Additional covariates such as age, comorbidities, drugs, cause of visit, and ECG measurements may also improve the accuracy of CVD risk predictions. The use of epigenetic markers and multi-modality networks are also areas of active research that show promise in predicting CVD risk.

In recent years, the medical community has become more interested in using ML algorithms to predict CVD risk using blood biomarkers. Through earlier interventions and better patient

outcomes, these algorithms have the potential to give clinicians more precise risk assessments. But several issues must be resolved to make these algorithms more dependable and efficient.

Data gaps are one of the key issues. By employing a data cleaning procedure that could deal with missing values in the dataset, Vanitha et al. study .'s from 2022 overcame this problem. This is a crucial step since the accuracy of the predictions provided by ML algorithms might be impacted by missing data. In addition, the study used clustering techniques to group biomarkers with similar measurement units, allowing for a more accurate data analysis. Another challenge is the differences in measurement units used for blood biomarkers. These differences can lead to inaccuracies in the analysis of the data and can make it difficult to compare results across different studies. The study by Vanitha et al. (2022) addressed this issue by aligning marker sequences and transforming the data into a base unit. This allowed for a more accurate data analysis and made it easier to compare the results with other studies.

Furthermore, Vanitha et al. (2022) demonstrated the potential of DL algorithms in predicting CVD risk using blood biomarkers. DL algorithms can provide more accurate predictions by capturing complex relationships between biomarkers and other variables, such as age and sex. The study used a DL-trained blood predictor, which could predict CVD risk with a high degree of accuracy. DL algorithms in CVD risk prediction can improve patient outcomes by providing clinicians with more accurate risk assessments. Nevertheless, there are still a few drawbacks that require attention in subsequent research. The assumption that patients who undergo "Examination within the Limits of the Norm" are healthy is one of the study's limitations by Vanitha et al. (2022). Although there may not be much of a CVD risk for these patients, other health hazards must be considered. It is necessary to do additional research to decide if these patients should be included in the analysis without any adjustments or with them.

Only a tiny percentage of samples provided a date of death; hence, further analyzing the study's assumption about alive/dead patients is necessary. If there are any notable variations between patients who pass away from CVD and those who do not, more research is required to confirm this. In conclusion, research into applying machine learning (ML) algorithms to predict CVD risk using blood biomarkers is ongoing. The study by Vanitha et al. (2022) has shown the potential of DL algorithms in this regard. The study's data-cleaning procedure raised the accuracy of the DL-trained blood predictor. More research is necessary to address the study's shortcomings, such as missing data and patient health presumptions. Overall, using ML algorithms to predict CVD risk has the potential to enhance patient outcomes by giving doctors more precise risk assessments.

Limitations

The study's assumption about "Examination within the limits of the norm" patients as healthy patients should be further investigated as it could be a risky generalization. Although these patients may have a low risk of CVD, they could still have other underlying health conditions that should be considered. For instance, a patient with a normal blood pressure reading may still have other risk factors that could lead to heart diseases, such as high cholesterol levels or diabetes. Thus, conducting a comprehensive health evaluation that considers a range of health indicators is important rather than relying solely on one health measure.

Only 284 samples in the study indicated a date of death. Hence the assumption about alive/dead patients needs further examination. The tiny sample size could skew the study

results, and it is challenging to infer any useful information on death rates from such a small sample size. It is also challenging to conclude the link between CVD risk and mortality because the study did not disclose the causes of death for these patients. The potential influence of environmental and lifestyle factors on CVD risk is a crucial element to consider. The study did not consider smoking, physical activity levels, and diet, which might have a big impact on CVD risk. Some study participants likely had a low risk of CVD because they led healthy lifestyles. In contrast, others with comparable test findings might risk more because of unhealthy lifestyle decisions.

The sample size for the study is also somewhat tiny and might not be typical of the overall populace. The study only included patients undergoing a comprehensive health evaluation at a single institution. It is unclear whether the study results can be generalized to other populations or settings. Moreover, the study did not account for potential differences in CVD risk among different age groups, genders, or ethnicities.

Another limitation of the study is the lack of long-term follow-up data. While the study provides important information about CVD risk in the short term, whether the results hold over longer periods is unclear. For example, some patients initially classified as having a low CVD risk may develop risk factors over time. Others with a higher risk may adopt healthier habits and reduce their risk. Finally, it is important to note that the study's results are based on a single type of health evaluation, which may not be suitable for all patients. The study relied on a comprehensive health evaluation that included a range of tests and measurements, but this evaluation may not be feasible or necessary for all patients. Some patients only need a basic evaluation, while others require more extensive testing based on their medical history, symptoms, or risk factors.

However, the prediction accuracy of ML algorithms can be impacted by missing values and variations in measurement units. Future research should ensure that the patient dataset is complete and contains more variables and ECG readings to overcome these problems. Furthermore, epigenetic biomarkers like DNA methylation should be researched in future studies since they may be able to offer useful data for predicting the risk of CVD. Ultimately, creating precise and trustworthy ML algorithms for CVD risk prediction can profoundly affect clinical practice by enabling early detection of those at risk for the disease and supporting tailored interventions to treat or prevent it.

It is important to look at the study's assumptions about healthy patients and mortality rates since they provide light on CVD risk and the value of a thorough health assessment. To further understand the connection between CVD risk and mortality, future research should consider various health indicators, lifestyle factors, and long-term follow-up data. Studies should also use a larger and more varied sample size to guarantee that the findings apply to a wider population. A complete and tailored approach to health examination is required to determine CVD risk and prevent cardiovascular disease effectively.

Conclusion

The study demonstrates the potential of ML algorithms for CVD risk prediction using blood biomarkers. The findings highlight the importance of data-cleaning processes and the inclusion of additional covariates and ECG measurements in future studies. Ultimately, developing accurate and reliable ML algorithms for CVD risk prediction can have significant

implications for clinical practice, enabling the early identification of individuals at risk of developing CVD and facilitating targeted interventions to prevent or manage the disease.

Accurate risk prediction of cardiovascular disease (CVD) is essential for efficiently managing and preventing this leading cause of global morbidity and mortality. Due to its potential to increase the precision of CVD risk assessment, applying ML algorithms for CVD risk prediction has drawn much attention in recent years. The study has shown that DL algorithms can forecast CVD risk using blood biomarkers. The study demonstrated that DL algorithms could precisely predict CVD risk using a combination of blood biomarkers and clinical variables using a large patient dataset.

The study's findings are significant, demonstrating that ML algorithms can be a powerful tool for CVD risk prediction. However, the study also highlights some important considerations for future research. One of the main challenges in using ML algorithms for CVD risk prediction is dealing with missing data, which is common in large patient datasets. The data-cleaning process used in the study effectively improved the accuracy of the DL-trained blood predictor. However, there is a need for further research to develop more effective methods for dealing with missing data in ML algorithms.

Additional variables and ECG measures are crucial factors to consider for future research. Age, sex, and blood biomarkers were included in the study as variables because they are key indicators of CVD risk. Smoking, diabetes, and physical activity are only a few of the significant clinical and lifestyle variables that can influence CVD risk. Future research that considers these characteristics may increase the precision of predicting CVD risk. ECG readings, which show data on the heart's electrical activity, can also be a helpful tool for estimating the risk of CVD. ECG measurements can find irregularities in cardiac function, which may signify a higher risk of CVD. Particularly in patients with normal blood biomarker levels, including ECG readings in ML algorithms may increase the precision of CVD risk prediction.

Creating precise and trustworthy ML algorithms for CVD risk prediction could ultimately impact clinical practice. Early detection of people at risk for CVD can help with tailored interventions to treat or prevent the condition. High-risk individuals, for instance, can be targeted for lifestyle treatments like quitting smoking and upping physical exercise. Pharmaceutical therapies like statins can also be employed to manage CVD risk in high-risk individuals. Clinicians can choose the best course of treatment by employing accurate CVD risk prediction provided by machine learning algorithms, which will enhance patient outcomes.

In conclusion, the study has shown that DL algorithms can predict CVD risk using blood biomarkers. The study emphasizes the significance of data cleansing procedures, the incorporation of extra variables, and ECG readings in future studies. The clinical practice and patient outcomes can both be significantly impacted by accurate and trustworthy ML algorithms for CVD risk prediction. Additional study is required to improve approaches for handling missing data and to expand the number of variables and ECG readings that may be incorporated into ML algorithms.

Chapter 6. Biological versus chronological age, longevity medicine. Introduction to senocardiology.

"Mitigating COVID-19 Mortality and Morbidity in China's Aging Population: A Focus on Available Medications and Future Developments." published in Bischof E, et al. Aging and Disease. 2023.

The COVID-19 pandemic, often called geropandemic, has put immense pressure on the global healthcare systems worldwide, leading to a rush in development and approval of medications for the treatment of the viral infection. The clinical trials on efficacy and safety had a limited spectrum on inclusion and endpoints because of the urgent need for fast results. The chronologically and biologically aged population are especially at risk for severe or lethal disease, as well as treatment-associated toxicity. In China, the growing elderly population segment has been a focus in public health measurements of COVID-19, guiding towards herd immunity with a mild variant, thus minimizing overall deaths and morbidity. While the COVID-19 pandemic has been now re-classified and the virus is weakened, there is a clear need for novel therapies to protect the elderly. This paper reviews the current safety and efficacy of available COVID-19 medications in China, with a specific focus on 3CL protease inhibitors and on the aging population. The current COVID wave in China demonstrated a significant impact on the elderly and the need for new drugs that are effective at low doses and can be used alone, without harmful side effects, generation of viral resistances, and drug-drug interactions. The rush to develop and approve COVID-19 medications has brought up important questions about the balance between speed and caution, resulting in a pipeline of novel therapies now moving through clinical trials, including the third generation 3CL protease inhibitors. A majority of those therapeutics are being developed in China.

Generation	Molecule	Countries	Active Companies	Highest Status	Administration route
The 1st generation 3CLpro inhibitors	Nirmatrelvir + Ritonavir (Paxlovid)		Pfizer Inc	Emergency Use Authorization in US, EU, Japan and China	Oral
	SIM-0417		Jiangsu Simcere Pharmaceutical Co., Ltd	Conditional approval in China	Oral
	GST-HG171		Cosunter Pharmaceutical	Phase II/III	Oral
	QLS1128		Qilu Pharmaceutical Co Ltd	Phase I	Oral
	ASC-11		Ascletis Pharma Inc	Phase I	Oral
The 2nd generation 3CLpro inhibitors	Ensitrelvir		Shionogi & Co Ltd	Emergency Use Authorization in Japan	Oral
	RAY1216		Guangdong Zhongsheng Ruichuang Pharmaceutical Co Ltd	Phase III	Oral
	FB-2001		Frontier Biotechnologies	Phase II/III	Inhalation
	PBI-0451		Pardes Biosciences Inc	Phase II	Oral
	EDP-235		Enanta Pharmaceuticals Inc	Phase II	Oral
	GS221		Grand Pharmaceutical Group Ltd	Phase II	Oral
	SYH2055		CSPC Pharmaceutical Group Ltd	Phase I	Oral
	SAL0133		Salubris Pharmaceuticals	Phase I	Oral
	STI-1558		ACEA pharma	Phase I	Oral
	PF-07817883		Pfizer Inc	Phase I	Oral
The 3rd generation	ISM3312		Insilico Medicine	Phase I	Oral

Table 1. Overview over 3CL protease inhibitors generation 1-3, and their respective country of discovery, highest status of clinical trials and administration routes.

COVID-19 and the urge towards approved therapies

The development of pharmacotherapies against the new SARS-CoV-2 virus since its onset in 2020 prioritized accelerated testing leading to rapid approval for use. Inevitably, there was only marginal focus on extended dosing, duration, safety, and efficacy endpoint measurements and inclusion of specific populations, such as those at high risk, pregnant, multimorbid, and aged persons. Especially the latter group proved to be most vulnerable and record the highest mortality rate. Despite this obvious fact, the current rapidly approved therapeutics for COVID-19 are not optimized or tested in the elderly. New generation of core therapeutics, especially 3CL-protease inhibitors, is urgently needed. Exclusion of the elderly in the clinical trials under pressure to recognize therapeutics, is accompanied with further deficiencies, e.g. despite quite early insights on sex and gender disparities in COVID-19, many clinical trials did not include those as biovariables. Meanwhile, it has been reported that males are affected more severely by COVID while females have a distinct immune response and are more prone to experience a cytokine storm and despite that fact that a more targeted optimization on dosing and onset of therapy is of great importance, as the male/female ratio becomes less with increasing age of the groups.

The initial accelerated approvals of COVID-19 drugs have been critical in ameliorating the evolving pandemic situation. This was particularly important in the early stages where there was only a rudimentary understanding of the virus' specific structure and pathomechanismsNevertheless, there is still insufficient data on the safety and efficacy of some of the drugs granted emergency approval, as well as reported sex differences as biovariable.

This article aims to address several key research questions and objectives related to COVID-19 and its impact on aging populations, particularly in China. A description of the relationship between COVID-19 and aging populations, highlighting the unique vulnerabilities and risks faced by elderly individuals exposed to SARS-CoV opens a discussion of the special need for safe and effective medications for vulnerable elderly populations, emphasizing the importance of developing treatments that are specifically tailored to their unique needs and challenges. Provides an overview of currently available drugs for COVID-19, specifically focusing on their application in China during the most recent surge of infection, highlighting the development pipeline of 3CL protease inhibitors, which have shown promise as potential treatments for COVID-19.

Aging and COVID – a vicious circle and China perspective

The COVID-19 pandemic initiated a number of debates and stimulated research in aging and related domains, while geriatric adjustments became more pronounced for a variety of subdisciplines at the frontline, and later also in disciplines covering the sequel of COVID-19 and Long-COVID. The "gero-pandemic" perpetuated investigations of potential targets of both aging and pathological processes, as well as their convergence.

Chronologically older adults, as well as those biologically aged individuals (e.g. due to comorbidities such as oncological or autoimmune diseases, altered microbiome etc.) , are primarily at danger of a lethal or at best a chronic protracted course of the disease, especially when there is no supportive *a priori* mounting of the vaccination by geroprotectors. And vice-versa, the infection itself accelerates the biological aging via various pathomechanism, such as inflammation (accelerated inflamm-aging). The same time, it has been well

documented for over 20 years that influenza-like infections, particularly influenza itself, result in increased cardiopulmonary morbidity and mortality. This is now also very plausible with the ongoing virus circulation of SARS-CoV-2. Thus, a protracted, chronic COVID-19 disease might contribute to accelerated aging. This is a vicious circle that can be broken with safe and effective treatment options.

China is experiencing a typical sociodemographic transition as has been the case in other countries alongside their development processes. This year was special due to a decline in its population growth for the first time in 60 years. At the end of 2022, the population of Mainland China was estimated at 1.41175 billion, which is 850,000 fewer than at the end of 2021. The annual birth number was 9.56 million with a birth rate at 6.77 per thousand, while the death rate reached 10.41 million, at a mortality rate of 7.37 per thousand, resulting in the population's growth rate of -0.60 per thousand, with a balanced male to female sex ratio of 104.69.

Since 2022, China's elderly population has grown rapidly. According to the data released by the National Bureau of Statistics, the newborn population in China was 367 million from 1962 to 1975. It is obvious that these people will grow old in the coming decades.

Nevertheless, the sheer size of China gives it substantial global significance. Governmental incentives might reverse the decline in the birth rate, and the ongoing efforts towards a healthy longevity of the citizens is contributing to a significantly lower rate of frailty and multimorbid dysfunctionality among those aged above 65 years, a majority of whom do not cease to be professionally engaged long after the legal retirement age. 209.78 million mainland Chinese citizens are 65 years old and over, accounting for 14.9% of the national population, which has increased by 9.22 million, or 0.7 per cent, since 2021.

While the COVID-19 crisis led to a reshaping of the society in various countries, the Chinese public health authorities anticipated and mitigated a potentially significant impact on the population of vulnerable older adults to avoid a massive negative impact on a physical, psychological, social, and economic level. Ageism was not to be expected, but the social isolation and unfavorable chronic health effects of much of his population required a salutogenic strategy, which was executed initially by strict prevention, positive pre-adaptation of people and the healthcare system, and ultimately a herd immunization with less aggressive SARS-CoV2 variants. In some other parts of the world hospitalized patients with a SARS-CoV-2 infection were intensively cared for due to COVID-19 at the beginning of the pandemic, especially with typical pneumonia. In contrast, the situation in China was similar to what occurred in 2021 in the West: a massively reduced mortality and morbidity rate due to vaccinations and hybrid immunity, especially during the emergence of Omicron and its variants.

China has experienced a continuous rise towards becoming one of the global leaders in biotech and innovation healthcare, including drug discovery and related clinical trials of new compounds. In addition, the number of internet hospitals alone is estimated at almost 2000 in number, delivering smoother access to healthcare for everyone, especially the elderly who are not in metropolitan areas and cannot physically reach a top-level hospital or pharmacy as well as for those who are exposed to infection risks at the physical healthcare institutions. Again, the elderly is the major part of this group, especially those with critical and complicated condition.

These factors enable China to bring promising COVID-19 therapeutics on the market and make them easily accessible to the masses.

Healthy Longevity Medicine and Science in China

One of the top priorities of the progressive healthcare policies and public health in the country which is benefiting massively from a fruitful and evolving biomedtech environment, is the healthy longevity. Healthy longevity medicine, defined as optimization of healthspan along lifespan, targeting aging processes, is aiming at mitigation and elimination of risk to develop age-related diseases, which lead to frailty, loss of productivity and massive burden on the medical resources. Various initiatives have been put in place to accelerate geroscientific research, as well as translation into the clinic and real-world programs. Coordinated efforts are facilitated through Healthy Aging Institutes and support of Smart and Internet Hospitals.

The Sequelae of COVID-19 and the need for rapid recovery

One of the core reasons to accelerate pharmacologic solutions to COVID-19, besides of the acute disease and reduction of severe conduct of illness, is the increasing knowledge of long-term post-recovery sequalae. Long-COVID and a variety of further solitary post-COVID residual symptoms have been described, and recently labeled as a parallel pandemic. Some of the post-COVID cases are multiorgan damage-related debilitating conditions, ranging as far as severe tremors, chronic pain, metabolic disturbances, and serious cognitive impairment. The pathomechanisms are still not fully understood and effective treatments remain underdeveloped.

One of the long-term conditions patients experience after recovering from COVID-19 is a new form of progressive pulmonary fibrosis that causes a severe decrease in lung capacity. This post-COVID pulmonary fibrosis (PCPF) was found in patients who were hospitalized due to COVID-19, but who were not always so seriously ill that they had to be ventilated or suffered lung failure - two well-known risks for the development of pulmonary fibrosis. The atypical disease course for pulmonary fibrosis caused tissue changes only four to twelve weeks after infection. Avoiding hospitalization and providing an accelerated recovery is thus of uttermost importance to curb the incidence of such cases.

Two of the hypotheses which address Long-COVID suggest that viral RNA persists in organ tissues and/or that tissue damage occurs during hospitalization following a severe course of infection. Significant reduction in patients' brain mass was recorded in the UK database even after a mild infection with COVID.

We need to understand the connection with SARS-CoV-2 more precisely and investigate whether treatment in the acute phase of the disease has an impact on the development of micro- and macroscopic damage in various systems. A large number of patients suffering from "long haul" syndrome are incapable of returning to and performing professional engagements, causing adverse socio-economic impacts. In both hypotheses, antiviral therapies are of major importance to swiftly eliminate the virus from the organisms and effectively and permanently block the replication to avoid Long-COVID which affected an estimated 45 million cases worldwide.

In China, Long-COVID has not yet manifested itself as a nationwide problem but it is expected to arise. Almost 60% of the COVID-19 patients in Wuhan still have a sequalae with increased pain and fatigue symptoms. Targeting the disease at its root with effective medication is likely to contribute to prevent Long-COVID.

Lessons on novel therapies from the COVID-19 wave in China 2023

In the recent wave of COVID infection in China in December 2022 and early 2023, Nirmatrelvir/ritonavir (Paxlovid) has been used in selected hospitalized patients with indications, according to the treatment guidelines, in which the drug had been integrated already in early 2022 ("Diagnosis and Treatment Plan for Novel Coronavirus Pneumonia (9th Edition)"). Surely it is a great advantage to have a targeted antiviral available during a surge of infections. But it became quite obvious that the clinical use of the drug was severely limited because the majority of hospitalized patients were elderly and multimorbid. Those with renal and hepatic insufficiencies were often treated with a lower dose at the discretion of the treating physicians (with even further reduced efficacy as per clinical observation). Official data on the efficacy of Paxlovid in China is still pending. However, single center experiences suggest that there is a rather diminished expectation that the drug will result in a significant improvement in the course of recovery. Nirmatrelvir is an inhibitor of 3CLpro, which can directly bind to the active site, inhibit the activity of the protease, and thus decelerate or cease the replication of the virus. However, the well-known rebound effect has been observed frequently, especially in the vulnerable population of the elderly. It is possible that these observations are biased due to the fact that most patients on the therapy were not meeting the actual recommendation of the Drug Administration, that is, to be initiated as soon as possible within 5 days after the diagnosis and the onset of symptoms, where Paxlovid is suggested for the treatment of mild to moderate COVID-19 pneumonia in adults with high risk factors (this includes age above 60 years) for severe disease. In the real-world, however, mostly only severely sick patients receive the drug only several days after the onset of the disease. Further practical considerations in frail elderly patients became apparent, e.g. polypharmacy (attention to CYP3A inducers, CYP3A-dependend meds including antihypertensives and statins), dysphagia (3 pills at the same time), little safety data regarding invulnerable patients prone to adverse reactions etc. The drug will not be covered by insurance in China beyond March 2023.

Domestic scientists conducted a randomized controlled phase III trial of a compound VV116 and Paxlovid, demonstrating a non-inferiority in regards to time to sustained clinical recovery among adults with mild-to-moderate COVID-19 at risk for progression. VV116 The study reported fewer safety concerns in VV116 as it is not interacting with drug transporters or metabolism. However, the study's main metric in this trial was similar to those which the FDA has previously approved for antivirals such as Xofluza and Tamiflu, that have been shown to alleviate symptoms faster than alternatives. For Paxlovid, in contrast, the EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) trial's primary endpoint has been their effectiveness in preventing hospitalization or death. The results showed a reduced risk of the above mentioned by 80%, as compared with a placebo, while also reducing the viral load on day 5 by 10 times. However, the speed with which symptoms are alleviated has not yet been studied in randomized trials. On December 30, 2021, VV16 has been approved for marketing in Uzbekistan. At present, two placebo-controlled trials are undergoing two phase III clinical studies. Paxlovid did not show any significant benefit in standard risk patients, while the corresponding trial EPIC-SR was terminated.

The State Food and Drug Administration conducted an emergency review and conditionally approved the Class 1 innovative drug Cenotevir/ritonavir (tablets) and Mindevir (Deuterium Remidevir Hydrobromide tablets). The above drugs are oral small-molecule drugs for the treatment of adult patients with mild to moderate novel coronavirus infection (COVID-19).

Cenotevir as the first domestic 3CL protease inhibitor (inhibitor of the RNA-dependent RNA polymerase) has been studied locally and compared with a placebo group, the treatment group had a significantly shortened time to recovery (1.5 days, and the subgroup of the high-risk group of severe illness was significantly shortened by about 2.4 days from the time from the first administration of 11 related symptoms to the complete asymptomatic state. After receiving a complete 5-day course of treatment of 0.750g of Cenotevir (0.375gx2 tablets) combined with 0.1g of ritonavir (0.1gx1 tablet), administered orally every 12 hours, the viral load was significantly reduced by 96%, and the time until testing negative under PCR tests was significantly shortened by 2.2 days. The detailed data of the study and a publication after peer review is awaited, while provisional reimbursement by insurance is available until the end of March 2023.

Mindevir is an oral nucleoside drug, non-covalently binding to the active center of the RNA polymerase, and mainly functions as a mutagen by increasing the frequency of transition mutations (G-to-A and C-to-U) in the viral genes. Preclinical studies have shown significant antiviral effects on the original and mutant strains of SARS-CoV2, including Omicron.

In China now, over 30 companies are developing and studying oral small molecule drugs for the treatment of SARS-CoV2. Those targeting 3CL protease inhibitors are listed in Table 1. The country is expanding the types and quantities of new drugs. All domestic COVID-19 medicines have been added to the national medical reimbursement list. Two newly approved homegrown pills — Xiannuoxin made by Simcere Pharmaceutical Group, and VV116 developed by Shanghai Vinnerna Biosciences — are temporarily covered up until March 31. Azvudine, the first domestic COVID-19 oral medication developed by Henan Genuine Biotech, was officially included in the last version of the national reimbursement list that was released on Jan 18. Three traditional Chinese medicine drugs and three herbal formulas have also been added to the list, the administration said.

3CL-protease inhibitors - three generations

Among the variety of proteases in the SARS-CoV2 virus, 3CL (3CLpro) protease is the main RNA processing protease in the virus' own code and is a crucial enzyme in the replication cycle of SARS-CoV-2. The mechanisms of action of these inhibitors vary, but they all work by targeting the 3CL protease and inhibiting its activity, which ultimately prevents the virus from replicating. One type of 3CL protease inhibitor is a peptidomimetic inhibitor, which mimics the structure of the natural substrate of the protease. This type of inhibitor binds to the active site of the 3CL protease and blocks its activity, preventing the virus from replicating. Another type of 3CL protease inhibitor is a covalent inhibitor, which forms a chemical bond with the protease and irreversibly inactivates it. This type of inhibitor is highly specific for the 3CL protease and has been shown to be effective in vitro and in animal studies.

A third type of 3CL protease inhibitor is a non-covalent inhibitor, which binds to the protease but does not form a covalent bond. This type of inhibitor can be reversible or irreversible, depending on its structure and mechanism of action.

Overall, the mechanisms of action of 3CL protease inhibitors involve inhibiting the activity of the 3CL protease, which is essential for the replication of coronaviruses, including

SARS-CoV-2. By preventing the virus from replicating, these inhibitors have the potential to treat or prevent COVID-19.

Early repurposing and compound discoveries focused on 3CLpro since HIV-specific proteases did not show promising inhibitory results in the early stage of the pandemic. Its inhibition results in the disruption of viral replication, and was thus considered a core therapeutic target from the early stages of the pandemic, leading to early suggestions for application in the prophylactic settings, as well as a rapid race by various companies toward repurposing existing drugs or the development of novel 3CLpro inhibitors for treatment purposes.

Several studies have shown that 3CLpro inhibitors can effectively reduce viral loads and improve outcomes in preclinical models of SARS-CoV-1 infection. SARS-CoV-2 has 79.5% sequence identity with SARS-CoV-1. However, the high mutability of the virus means that resistance to 3CLpro inhibitors emerge quickly, highlighting either early combination therapies and ongoing monitoring of antiviral resistance, or a fully novel generation of the protease inhibitors that can overcome the challenges as single agents.
Table 1 summarizes the three generations of 3CLpro inhibitors, their current stage of clinical development, and their route of application. Ten out of 16 protease inhibitors originate from China, five from the USA and one from Japan. All (other than FB-2001 (II generation, inhalation)) are oral compounds. Oral routes are preferred in general settings, while inhalations and intravenous administration (still not feasible) are reserved for acute care settings, such as intubated patients without nasogastric tubes.

The first-generation compounds are limited by several factors, such as the need for co-administration with ritonavir (or another CYP3A4 inactivator) to maintain the drug plasma concentration. However, such a co-compound may stimulate drug-drug interactions in clinical usage. Ritonavir is contraindicated to be used concomitantly with a wide range of drugs that are highly dependent on CYP3A4 for clearance and for which elevated concentrations are associated with severe adverse reactions, such as pulmonary arterial hypertension, oncological, and arrhythmic medications. At the same time, co-administration with potent CYP3A4 inducers is also problematic, since significantly reduced 3CLpro inhibitors or ritonavir plasma concentrations may be associated with the potential for loss of clinical response and possible acquired resistance. In addition, co-administration of ritonavir in HIV patients may lead pose a risk of developing HIV-1 resistance to HIV protease inhibitors.

The second generation of novel 3CLpro inhibitors can probably achieve single agent clinical usage without a ritonavir boost, but the local concentration of the compounds may be reduced and thus inhibition impaired by the multidrug P-gp transporter (Fig.1). Reduction of inhibitor concentration leads to viral mutant selection and possible acquired resistances, just as it was reported in anti-HIV-medication. There are reported efforts to develop approaches to minimize the effect of P-gp in drug transport and to increase the bioavailability of the orally administered drug by use of P-gp inhibitors, specifically targeting P-gp efflux. However, a single agent medication is always preferred in the real-word and clinical setting.

The third generation 3CLpro inhibitor could achieve single agent status in clinical usage without a ritonavir boost and inhibit the target in an irreversible manner. The inhibitory activity of a novel covalent irreversible core has not been impaired by the p-gp transporter. In addition, the new generation 3CLpro inhibitor could counter potential acquired viral resistance found in the clinic (Fig.1). The choice of administration route for a 3CLpro inhibitor needs to take into account multiple factors, including the timing of treatment, the stage of the COVID-19 illness,

and the bioavailability of the antiviral. Currently, there is a need for a more convenient method of administering COVID-19 medication to early stage, non-hospitalized patients, as well as for pre-exposure and post-exposure prophylaxis options. An ideal solution would be an oral delivery option for early-stage disease management or for pre-exposure/post-exposure use, at a high oral bioavailability (first-generation 3CLpro inhibitors PF-00835231 and GC-376 showed a bioavailability of 1.4% and 3%, respectively in rats). Third generation compounds hold a promise to meet these criteria. As of February 2023, one compound of the third generation entered clinical trial stage. This is a covalent irreversible 3CLpro inhibitor developed with assistance of artificial intelligence.

Discussion

Although the rapid development of COVID-19 medications represents a significant success for science and public health, there are also some crucial limitations, which can and should be tackled as the urgency of the pandemic subsided. First, the middle and long-term safety was not possible to be studied, long-term negative effects cannot be fully excluded, as pharmacologa were repurposed or developed and tested within a shorter time frame than usual. Although clinical trials show that most of the drugs have a good safety profile, further monitoring and follow-up must be conducted. Secondly, the availability is also limited, since a rapid development and approval does not automatically translate into a broad available. The production and distribution remain a challenge and can lead to shortages and delays.

Thirdly, the effectiveness against new virus variants is limited under the pressure of the rapid development: as the virus constantly mutates, there is a possibility that the current medications may be less effective against newly emerging virus variants. All the three aspects are specifically affecting the elderly population, since they are the most vulnerable ones to suffer from side effects, inequitable distribution:
Economic burden: The development and production of vaccines require significant resources, which can result in high costs. This can pose challenges in financing and distributing vaccines, especially in resource-poor countries.

Conclusion

COVID-19 infections manifest in vulnerable populations, of which biologically aged patients experience severe disease course and have worse prognosis. Older adults who are mostly excluded from clinical trials, require safe and efficacious medication which won’t interact with chronic diseases and chronic therapies.

As COVID-19 becomes progressively endemic, newly developed compounds can tackle several shortcomings of the currently approved repurposed medication, such as drug-drug interaction, limited effectivity or reproductive risks, low safety profiles and broad strains coverage. Second and third-generation 3CLpro inhibitors represent a promising approach in the development of effective treatments for SARS-CoV-2 infections. Current antiviral protease kinase inhibitors were of a great value in the acute pandemic setting. The importance of having very safe and selective therapies that do not harm the elderly should now be the priority in COVID-19 medication development.

Further research is needed to fully assess the clinical efficacy and safety of these inhibitors, especially in mild/moderate cases in the outpatient setting. For future studies and research efforts, it is core to consider COVID-19 an infection which will continue to cause waves of

infection, just like other respiratory viruses. Since elderly and biologically aged individuals will remain a vulnerable population, while demographics is sharply shifting globally towards a negative growth, it is important to focus on mitigating the overall disease burden and sequelae with effective, minimally toxic and elderly-suited medications, beside of prevention and advanced care planning.

Tackling COVID-19, will also have an impact on healthy longevity: an increase in disability-free life expectancy with a higher quality of life, without significant limitations in daily activities.

The study demonstrates the ongoing progress of medicine, as people in this age group, especially those over 70, have accompanying illnesses or risk factors that require treatment in over 50% of cases.

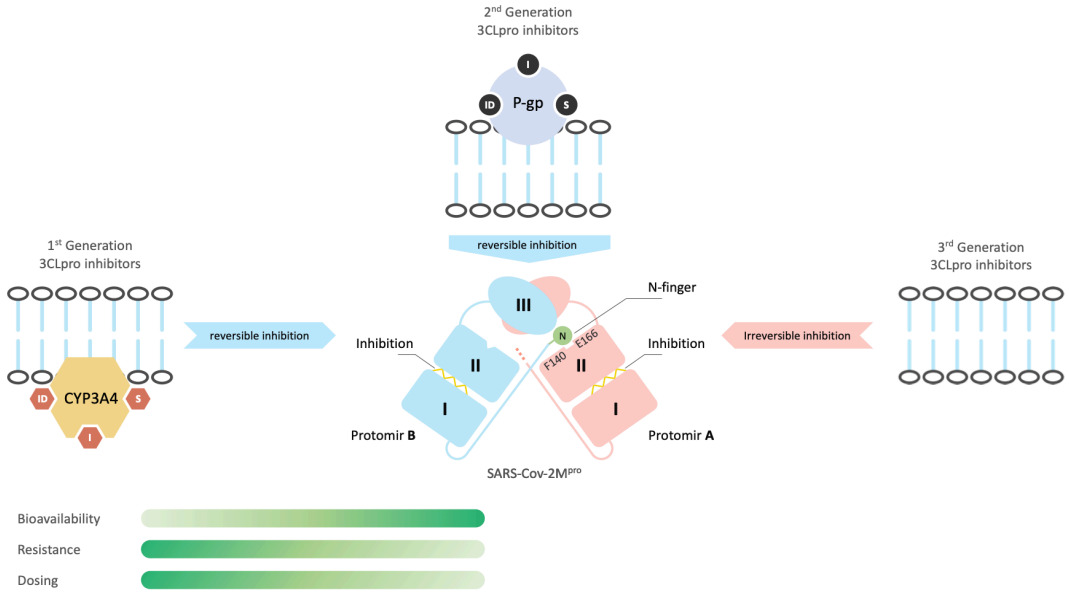


Figure 1. Key differences in properties of 3CL protease inhibitors of SARS-CoV2: first generation is usually combined with a CYP3A4 inhibitor in order to allow a sufficiently high plasma concentration of the active drug; this leads to an increased drug-drug interaction. The bioavailability is limited, the dose must be higher than desired and resistances develop rapidly in HIV patients. Second generation is assumably affected by the P-gp interaction, which also leads to resistance development against new viral variants. The third generation hold potential for a single agent which acts irreversibly (as opposed to generation 1 and 2), single dose (high bioavailability) and less clinical resistance.

Chapter 7. AI in longevity medicine.

"Artificial intelligence in longevity medicine." Zhavoronkov, Li Kai-Fu, Bischof, published in Nature Aging. 2021.

Abstract

Recent advances in deep generative reinforcement learning enabled the development of artificial intelligence (AI) systems that outperform humans in many tasks and have started to empower scientists and physicians with new research and clinical tools. In this article, we discuss how recent applications of AI to aging research are leading to the emergence of the field of longevity medicine.

Age is the universal feature shared by all living beings. While the rate of aging may vary among individuals and species the time elapsed since birth is a strong predictor of health status and mortality. Targeting aging may extend the average life expectancy more substantially then prevention or treatment of the individual diseases. However, within the established drug discovery and development framework, pharmaceutical companies are still searching for compounds and interventions for the treatment of individual chronic diseases such as cancer, cardiovascular or pulmonary diseases. Current biomedical research aims to identify the underlying mechanisms and molecular targets specific to a disease in order to modify the disease, treat its symptoms or cure it. Once a drug is approved, the medical community is obliged to follow a specific and defined protocol that is mostly targeted towards treatment of a specific disease with a specific drug or a combination. Rarely, however, do clinicians prescribe an off-label drug - even when there is evidence that it may be effective in the treatment of another disease to avoid possible malpractice claims and to avoid the possible side effects. While evidence-based medicine and has been overwhelmingly effective for reducing overall mortality over the past half century, it has also increased the economic burden of disease in developed countries due to the resulting extension in lifespan without a concomitant increase in healthspan. Since aging plays a key role in the onset and progression of many human diseases and affects all organs of the body, many chronic conditions manifest themselves simultaneously as comorbidities later in life. Combined with global trends in population aging, this apparent victory of modern medicine to extend our lives has led to an explosion in healthcare costs and has failed to concomitantly improve wellness and quality of life for older adults.

The advent of AI in biomedical research and medicine

Preventive, geroscience-based measures to treat aging at the organismal level, the root cause of most non-communicable and a major contributor to susceptibility and vulnerability towards communicable diseases, would provide a more substantial benefit than reactive therapeutic approaches targeted at a single disease or organ since those do not contribute to a significant improvement in healthspan. It is often ignored that disease-focused therapeutic approaches do not remarkably extend lifespans. It has been estimated that the complete elimination of a single fatal disease such as cancer in the US would merely lead to a 2.3 year population increase in life expectancy at birth and a 1.2 year increase at age 65 since the majority of overall mortality is due to age-related diseases and the abovementioned multimorbidy. Old biological age is not recommended to be reported as an official cause of death, but it remains the main reason why older adults die worldwide.[2] Therefore, combining the prevention and elimination of chronic diseases by adding a geroscience approach to

routine clinical care would yield the best outcomes in that it would promote both a long and healthy lifespan.

However, understanding the aging process requires longitudinal monitoring of millions of parameters in many different types of datasets that change very slowly during the human life course, and distinctly in genetically and socio-culturally diverse populations. While humans can be trained to accurately predict age and explain features leading to their predictions, using facial images for instance, and even to propose corrective actions, no human doctor can do this on multiple different biological data types, such as blood tests or gene expression data. Fortunately, the task of finding complex patterns in large volumes of longitudinal data is where modern artificial intelligence (AI) demonstrates unique, often spectacular performance. Since 2014, AI systems have outperformed human experts in multiple areas, including image recognition, knowledge quizzes, video games, language translation, and many other tasks. Most of these achievements have been made possible by various advances in deep neural networks trained on large data sets using high-performance computing. importantly, deep generative reinforcement learning has been successfully used in a broad range of biomedical applications (Figure 1a) ranging from drug discovery to prediction of clinical trial outcomes and personalized medicine.

AI-powered tools enable longevity medicine

Deep learning (DL) was a breakthrough for AI research, allowing to train deep neural networks (DNNs) on massive longitudinal data sets, which were previously almost impossibly difficult to comprehensively mine and interpret in the longevity arena. There is no consensus on how to define human "biological age" but the term usually refers to a measure that is more predictive of mortality, diseases or frailty than chronological age, that changes in response to geroprotective interventions, and can that track some of the biological hallmarks of aging such as the promotion of cellular senescence by smoking for instance. DL was instrumental in establishing Deep Aging Clocks (DAC) that not only generate estimates of an individual's biological age state (Figure 1b). Using AI-powered tools such as DAC, clinicians should be able to more precisely assess and monitor individual health risks and tailor appropriate interventions or changes in lifestyles for a specific person. We argue that DAC should become an essential part of the physician's tool kit, enabling AI-supported recommendations to promote long and healthy lives. Other DL-based solutions that outperform humans, such as radiological image analysis algorithms for early cancer or aneurysm detection, dermatologic testing, can provide further benefits. In this context, we define longevity medicine as a branch of precision medicine that is specifically focused on promoting healthspan and lifespan and that is powered by AI technology. AI-powered longevity medicine will facilitate the discovery of drug targets for specific individuals, the identification of tailored geroprotective interventions, and aging and longevity biomarkers to enhance the study of aging and disease trajectories, and the identification of interventions that may help slow down or even reverse aging-associated biological, physiological, or psychological processes. As recent advances in longevity biotechnology and AI are starting to percolate through clinical research and clinical practice, physicians will increasingly need to navigate through various AI technologies and applications, including those that may be relevant to the nascent field of longevity medicine.

Opportunities for longevity medicine in clinical care and the longevity industry

In order for longevity medicine to be referred to as a branch of medicine, it needs to be practiced by physicians. Clinical practice requires clinical protocols, diagnostic and treatment guidelines, with defined outcome measures, biomarkers and medications approved by national regulatory authorities, such as the FDA. However, to develop at least preliminary clinical recommendations for interventions in longevity medicine, aging needs to be monitored and treated as a medical condition, with designated studies performed to demonstrate both efficacy and safety of specific interventions. At present, there is a growing number of AI-based tools that give access to relevant health parameters such as a patient's biological age, that utlize a variety of "omics" data and present different dimensions of a patient's state of health, rate of aging, and inform prognosis. In order for these tools to be adopted by clinicians and accepted by the medical community, they need to be integrated into the current framework of clinical practice, ranging from primary, through secondary prevention, treatment, and monitoring. Such integration requires the convergence of modern AI and modern medicine, through a symbiotic collaboration between clinicians, geroscientists and AI researchers. Physicians should be encouraged and have the chance to be involved in AI-based longevity research. At the same time, AI-powered longevity biotechnology and AI-based biomarker-driven science should be promoted and seek close clinical and meta-clinical collaborations. Doctors first need to have the access to tailored, validated and credible education on AI-based biogerontology sciences, such as accredited courses that would further allow to build a longevity physicians' network and ultimately create a separate medical discipline. A basic knowledge of AI-driven geroscience is essential to bring relevant scientific discoveries into trials and study outcomes to the clinic.

Beyond academia and regulatory authorities, industrial involvement will also be important. Like in the early days of the Internet when we witnessed the appearance of mobile technologies, instant messaging, and social networks, we expect to see more venture-backed groups of expert enthusiasts starting businesses in this emerging area. These businesses will provide AI-based tools, such as DACs to track aging, that will help build evidence-base knowledge from the data provided by and to clinicians, as well as from high-end clinics working with self-funded clientele interested in extending the period of healthy productive life.

As the field of longevity medicine develops, we must also take into consideration its potential impact on health equity. For all branches of medicine, there is a great ethical concern stemming from major inequities in health outcomes due to various factors, including socioeconomic status, geopolitical position and ethnicity. One could assume that the access to longevity medicine solutions will only be available to wealthy individuals and thus deepen the health disparities. However, longevity medicine is partly equipped with a low cost and minimally-invasive arsenal (e.g. trackers, DACs, etc.) that is readily applicable to public health problems. We therefore believe that there is a potential for longevity medicine to reduce health inequalities. Genetic testing already proved that a democratization of specialized precision medicine testing is possible. Similar developments are to be anticipated for other -omics and even imaging techniques.

Longevity research in academia and in the biotechnology industry should also benefit from large pharmaceutical companies willing to employ tools for clinical trial enrollment and monitoring, and from life insurance companies interested in providing additional services to customers desiring large payouts at the end of their lives. Ultimately, such collaborations will also encourage the creation of industry groups or branches within regulatory agencies that

will define and establish common clinical practice and industry standards and regulations required to guide the future of AI-based longevity medicine.

Conclusion

Given the rapid progression of AI-based experimental longevity medicine, it is now time to catalyze its translation to common clinical practice. This transition will bring novel solutions to patients and healthy individuals. Longevity medicine is also an opportunity for multidisciplinary collaborative work of thus far often distinctive players to transform public health into public healthy longevity.

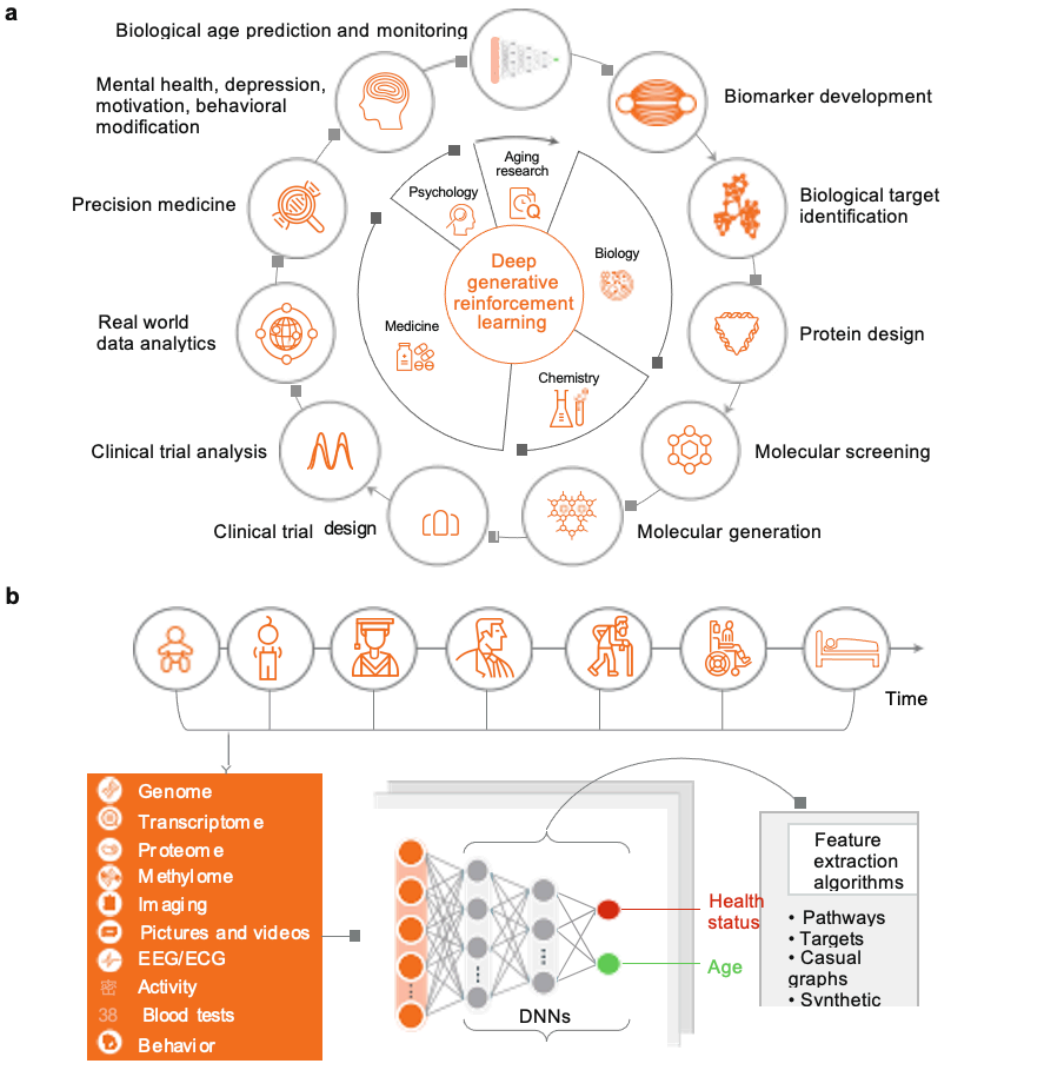


Figure 1. Artificial Intelligence in Longevity Medicine.
a. Deep generative reinforcement learning for every aspect of healthcare at every level of system organization. A multitude of DL application in the clinical practice is represented. b. Deep neural networks trained on longitudinal data of healthy subjects and patients with diseases learn the difference between aging and disease and have the potential to lead to applications for risk prediction and identification of optimizable factors, with the ultimate goal of positioning individuals in their optimal performance biological age range.

Chapter 8. AI in Medicine

Book chapter: "AI in Medicine." Radenkovic, Zhavoronkov, Bischof., Springer Nature 2022

Abstract

Since 2013 deep learning systems, a form of artificial intelligence (A), outperformed humans in image, voice, and text recognition, video games, and many other tasks. In medicine AI has outperformed in dermatology, ophthalmology, and several areas of diagnostic medicine. Since the first publication of aging clocks based on methylation data in 2013 by Horvath and Hannum, AI techniques were used to predict human age, mortality, and health status using blood biochemistry in 2016, and later using transcriptomics, proteomics, imaging, microbiome, methylation, activity, and even psychological survey data. Today, these deep aging clocks (DACs) are being used by the research physicians to evaluate the effectiveness of longevity interventions, clinical trial enrollment and monitoring, risk profiling, biological target identification, and personalized medicine. The advent of data type-specific and multi-omic DACs allowed for the nascent field of aging clock-driven preventive and regenerative medicine, referred to as longevity medicine to emerge.

Introduction

Over the past decade, we witnessed unprecedented advances in the field of biogerontology, and the massive convergence of biotechnology, information technology, AI, and medicine. The birth of longevity medicine, which integrates the latest advances in many of these fields of science and technology is not surprising, but rather embraced by progressive clinicians, scientists and patients. Longevity medicine is advanced personalized preventative medicine powered by deep biomarkers of aging. This domain is extremely novel - aging clocks were first published in 2013 by Steven Horvath et al. and deep aging clocks first published in 2016 by Alex Zhavoronkov et al. Nevertheless, it became one of the most important areas of precision medicine. What started with a symbiotic effort of mathematics and biochemistry, evolved to an artificial neural network approach - deep learning. The method is used to develop deep age predictors with the potential to accelerate research and clinical translation of causal relationships in nonlinear systems. Applying the clocks in the clinic may allow clinicians to determine the efficacy and efficiency of interventions, prognostic and preventative measures. A tailored longevity medicine education is incumbent in order to train clinical experts in longevity medicine, who will play a crucial role, since the universal process of aging renders every human a patient of longevity medicine, therefore preventing the multimorbidity characterized by old age, including physical and/or mental limitations. Drug therapy poses a further problem of multimorbidity. This is because older people often take many different medicines and this can also increase the number of undesirable side effects.

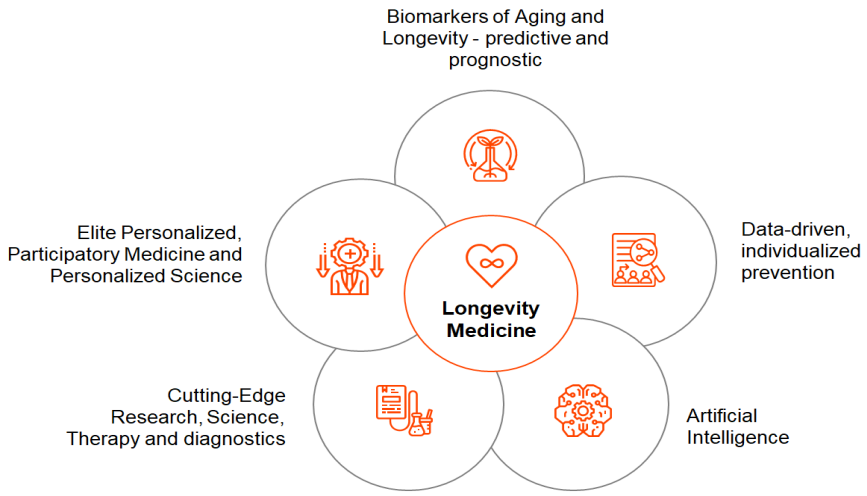


Figure 1. The technologies enabling the field of Longevity Medicine

Longevity Medicine is suited for advances in machine learning for numerous reasons. Longevity is affected by a large number of intrinsic factors such as genetics and epigenetics, and by extrinsic factors such as medical history, diet, exercise, socioeconomic determinants of health, and geolocation. Additionally, the effects of these exposures summate over the course of a lifetime affecting, among other factors, development or progression of age-related disease. Collating this data on biomarkers and drug candidates using a trial by trial basis conducted on sufficiently large patient or participant cohorts over long term follow-up is resource intensive and unpragmatic. For such heterogeneous and complex data, machine learning is a well suited candidate both to identify suitable biomarkers in advance of physical trials, and to do so with higher accuracy than traditional statistical analysis.

A quintessential theme of a machine learning algorithm is endpoint selection. Actual age and clinical outcomes such as the development or progression of age-related disease provide a limited view of one's age status. Biological age, which more closely explains age status in the context of one's physiology is a measure that focuses on progression through aging instead of actual age. Surrogate biomarkers of biological age, termed deep ageing clocks have been shown to estimate disease mortality, discern the age of developmental tissue, and estimate time remaining before onset of age related pathologies. The utility of these tools is often based on multi-omic or panomic databases with extensive measures generated on trillions of data points. The interpretation and analysis of these datasets requires advanced techniques for which machine learning may be better suited over traditional methods.

It is often difficult to train an accurate model based on a single dataset as this makes the model prone to bias and it may not have the desired complexity given the project aims. Additionally, databases are generated inter-institutionally with different protocols and specifications across potentially diverse biological features, which makes some of them prone to having gaps in data provision, necessitating the imputation of missing or unknown values. Access to multiple datasets will negate these effects, but this is practically near impossible due to inherent issues of confidentiality and governance surrounding sensitive medical data. Therefore, techniques like federated learning that train unified models across multiple databases without sharing the data sources would overcome some of the strong barriers around patient confidentiality. This may also bridge together wisdom gained from the academic and medical fields with wisdom

generated from data collected in the private sector, which is traditionally difficult due to large conflicts of interest.

The Advent of Deep Aging Clocks

Many biological characters have demonstrated broad correlations with chronological age, including telomere attrition and racemization of amino acids in proteins. The primary progenitor, however, in the advent of aging clocks is what would become known as the 'Epigenetic Clock'. In 2013, Steve Horvath et al. established significant correlations between CpG methyl groups and chronological age, suggesting that this deep aging clock demonstrated the cumulative effect of an epigenetic maintenance system. This has led to significantly increased attention in the fields of longevity, biogerontology, biomarkers of aging and the role of machine learning in consolidating these data into precise and coherent models.

What was not well established were geroprotective interventions that could reduce the rate of aging in the novel epigenetic clock, and whether these could translate to the actual lengthening of both healthspan and lifespans. Hence, research proliferated utilising haematological biomarkers indicative of morbidity and mortality putatively involved with aging. This would include a haematological, biochemical clock developed by Putin et al. Through the use of deep neural networks (DNN), the group were able to train AI to produce a robust model utilising 41 factors that could predict one's chronological age with impressive accuracy ($R^2 = 0.82$, MAE = 5.5). The benefit of employing DNN architecture in model generation in this instance was that it was able to recursively remodel and account for non-linear associations among features. Importantly, they established for their model the five most important markers for predicting human chronological age: albumin, glucose, alkaline phosphatase, urea and erythrocytes. What was particularly useful for this deep aging clock was that interventions that could ameliorate the biomarkers used in the model were already well established, and hence, some of the first truly geroprotective interventions were identified.

Indeed, the advent of deep biomarkers of aging has led researchers to target different loci in human and animal biology. For example, researchers as early as 2015 were investigating the role of gene expression through relative quantitative changes in mRNA presence for particular genes and correspondent proteins. Using a cohort of 14,983 individuals, they identified 1497 genes that are differentially expressed with chronological age and used this to develop a clock with a 7.8 year MAE. Importantly, they established that individuals for whom transcriptional differentiations departed above the model's slope exhibited biological features associated with aging, notably, blood pressure, cholesterol levels, fasting glucose, and body mass index. They also established that their gene panel was enriched for the presence of potentially functional CpG-methylation sites in enhancer and insulator regions that associate with both chronological age and gene expression levels, therefore discovering a mechanism by which the epigenetic clock (DNAm age) could behave as an actor in the development of morbidities with age.

Another transcriptome aging clock was produced in 2018 by Mamoshima et al. In this instance, they employed the use of DNNs to predict chronological age from 545 gene expression profiles in skeletal muscle of healthy individuals. Among currently published transcriptomic clocks, this clock is the most accurate, demonstrating an MAE of 6.24 years, which may indicate that transcriptomic age prediction requires more complex machine learning techniques than those commonly used in DNAm clocks.

Federated Learning for Biomarker Discovery and Development

One's longevity is dependent on a large number of covariates such as dietary habits, exercise levels, and socioeconomic determinants of health. As a result, unbiased long-term trials are used to identify or repurpose new or existing agents that contribute towards greater longevity. For example, the Targeting Aging with Metformin (TAME) trial investigates the use of metformin to delay development or progression of age-related chronic disease in 3000 participants aged between 65-79 for 6 years. Ageing is chronic and develops in conjunction with long-term environmental exposures. Thus, it may be a further 10 or more years post-trial for the clinical endpoints to appear in participants before for the impact towards longevity can be fully evaluated.

The long time-to-event in trials may come at high cost for longevity drug discovery. Machine learning (ML) algorithms may be employed to generate advanced accurate predictions into the efficacy of interventions, particularly for repurposing existing medicines. Data on existing medicines is collected in electronic health records and the generation of research repositories. This applies especially for commonly prescribed medicines such as metformin.

ML is well suited to the complex variability of human studies and is already being applied for fields such as drug target discovery and protein folding, but a limitation into their application is often a shortage of usable data. Large panomic databases are generated to provide ample numbers of data points for medical research, such as the UK BioBank comprising 500,000 participants. Despite the large number of participants, when variables of interest are selected such as a given disease, and when covariates such as ethnicity are stratified, the usable numbers of participants may be insufficient to train an accurate machine learning algorithm. Furthermore, curated datasets may also have biases stemmed from processes such as participant acquisition. To address these limitations, it may be tempting to consolidate different datasets in order to have a sufficiently sized dataset including all variables of interest generated from a variety of sources to counter bias that would be present in a single dataset.

Medical data is highly sensitive and there are many regulations over how it is used. Anonymisation by removing personally identifiable information is generally insufficient to surpass this barrier making it practically difficult to combine medical datasets. Another reason why medical data is unstandardised is that generating shared datasets is time intensive and may be costly.

Federated learning is a ML algorithmic methodology which was created for collaborative model generation where the learned wisdom is shared but the data is not shared between repositories. This means that a single model can be generated without breaking data governance over a large number of datasets. Federated learning by design consists of using a central server to distribute nodes for each database source behind the owner institution's firewall. The local nodes train the model and return it to the central server for aggregation and redistribution to the local nodes. Split learning, a type of federated learning uses a peer to peer design, where there is no central server and the individual nodes share their trained models, aggregate them locally, and redistribute them with each other. These protocols for both designs are repeated until the training is completed. Federated learning performs faster than split learning as the clients generate models in parallel, but split learning has improved privacy as the architecture is split between the clients and the server but is more computationally intensive on each local node.

In addition to federated learning, other methods can be employed either as alternatives when federated learning is unavailable, or to help validate a federated learning model. The bias and variability of single non-federated models trained on small datasets can be compensated against using traditional statistical methods. ML models on small data can have a tendency to overfit training data and may not interpret unknown data accurately, but using models such as logistic regression on expert selected features, and selectively removing outliers may improve the efficacy of the model. This methodology may be further optimised by running multiple models and generating a weighted average.

Longevity physicians - emerging specialists and the need of a tailored education

With the progress in geroscience and biogerontology, as well as in AI-based diagnostic and therapeutic tools, longevity medicine as a field has been increasingly in demand by educated patients. For longevity medicine to become an organic expertise area or, preferably, a specialty, just as oncology or cardiology, it needs to be practiced. This in turn implies a solid education of physicians, allowing them to not only acquire the necessary fundamentals, such as hallmarks of aging, but also to rapidly internalize and apply related research outcomes and tools.

Furthermore, as to develop and adequately practice longevity medicine, an ongoing continuous learning is required, encompassing the likewise rapidly evolving areas of precision, prevention, and functional medicine. Ultimately, educating physicians in longevity medicine is one of the most challenging endeavors, requiring a structured, meticulous approach of interdisciplinary educators. Overall, physicians' continued education is still inadequate to meet the challenges and opportunities of longevity medicine.

Current medical training does not include AI, not to mention machine or deep learning. The AI-enforced tools for early diagnostics and prevention of communicable and non-communicable (NCD) diseases are thus unpopular among the majority of physicians. The so-called ‘Millennials’ are increasingly exposed to innovative solutions in medicine and to active patients’ enquiries. Facing a lack of structured, physician-oriented, conceptualized curricula, this new generation, with a tremendous potential for global healthy longevity, is deserting and often diffusing away from the field. At the same time, a growing interest in longevity medicine is observed among related fields including biotechnologists, biologists, geroscientists, as well as among the general public, pharmacologic companies and target industry groups. Interestingly, the latter two demonstrate an extremely high interest in growing a longevity physician community, while specific approaches to incentivize specific educational approaches (building of certified courses, curricula, accreditations) that require significant resources. As long as academia will not sufficiently support such endeavors, the educational initiatives remain grandly scattered, in a form of individual or lecture series given by mostly non-clinical experts. In addition, despite the benefits longevity-focused medicine has to offer, currently, only its fragmentary aspects are available within the certification system: lifestyle, holistic, integrative medicine, or geriatrics. Extremely time-limited clinicians rarely can accommodate extracurricular educational activities in their schedules. Currently, we mostly face reactive medicine, managing diseases rather than mitigating risks towards pathogenesis. Since most health care systems are fragmented, specialists are often dispersed and comprehensive patient care is suboptimal. Most importantly, there is extremely limited access to the practitioners who have self-initiated their education into longevity sciences, follow the rapid development of various diagnostic and monitoring solutions, and implement these from the longevity standpoint.

The paradigm of longevity and healthy aging as the top priority will greatly impact the primary, secondary, and tertiary prevention rates; it is essential that doctors have the access to well-structured and practitioner-friendly course contents. Development of such courses is only possible through interdisciplinary efforts that would generate contents and contexts teaching both the fundamentals, as well as ways of how they can be implemented in the clinical practice.

Education is the foundation for longevity health findings to be implemented in the daily practice for patients and in preventative settings. It decimates the knowledge gap between physicians and non-clinical longevity experts (bio-/gerontologists, AI- and computer scientists etc.) and reduces stigmatization of longevity medicine being falsely dismissed as a highly anecdotal movement towards life prolongation. In contrast, longevity medicine is a highly scientific approach towards the extension of a healthy and productive lifespan, with an AI-based precision approach using measurable markers of aging.

An accurate physicians' education in longevity medicine must aim to clearly demonstrate current medical practice, which evaluates and optimizes the parameters of patients within the reference range for their corresponding age groups from truly personalized medicine based on large data. Even if an age group is selected based on a variety of further variables, such an approach is at most a personalized one, but not a precise or individual one. Longevity medicine brings together the best practices from various biomedical disciplines and AI to evaluate the patient's biological age throughout his/her course of life in order to reduce the gap between the current and the parameters of maximum physical performance (based on a calculated ideal biological age). A longevity physician is thus required to be able to not only apply measurements of aging clocks, but also to then (with AI-assistance) identify ways to reduce the gap between the current and the optimal biological age. These ways include, at the moment, non-invasive lifestyle modifications (e.g. physical activity, intermittent fasting, circadian rhythm readjustment) or geroprotective supplements, and anecdotal invasive methods. It is apparent that these approaches will require ongoing data collection in order to customize and improve protocols, as well as individual AI-supported recommendations.

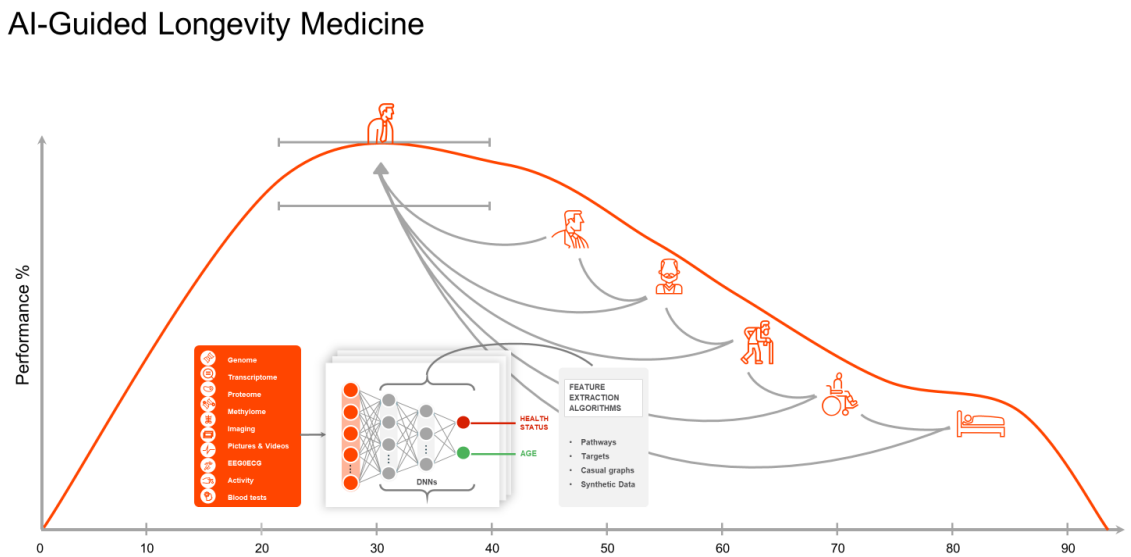


Figure 2. How human performance changes with age. Panomic technologies integrated with deep neural networks can interpret this and identify geroprotective pathways

Only in the year 2020, a global interdisciplinary team developed the first official Longevity Medicine for Physicians course, covering topics of biogerontology, machine learning, biostatistics, differential diagnosis, programming, molecular biology, immunology, geroprotective interventions, drug design, healthcare organization, and others, as well as providing an overview of clinical applications of recent advances in aging research, skills to evaluate the validity of biomarkers of aging and other biological age testing systems, and knowledge of the available longevity therapies to tackle diseases that are mostly based on senescence-related processes in the organisms. It additionally bridges discrepancies in awareness and information on advances in research, while bringing practicable examples of implementation into clinical practice. The unprecedented increase in the percentage of people over 65 years of age and corresponding increase in the illness, social, and economic burden associated with aging requires us to advance our understanding of the ageing process and how to tackle those processes and provide the care needed. This and similar initiatives will ultimately lead to major beneficiations in healthcare systems as paramount, effective management of diseases, since progress in longevity and biogerontology research will likely increase the healthy productive lifespan and the number of years of government support in old age. It is therefore incumbent to educate physicians about the most recent parameters that can be applied to established models of prevention of diseases by tackling and applying biogerontology advancements. As such, they will be linked to economic growth via biomedical progress rate, the rate of clinical adoption, and the rate of change in retirement age.

Aging is a complex multifactorial process leading to loss of function, causing multiple NCDs, rendering prone towards CDs, and premature mortality. There are many theories explaining the origin of the overall process, cause and effect relationships between different processes and systems, including aging of the immune system, inflammation, fibrosis, mineralization of connective tissue, cellular senescence, wear and tear, and many others. In addition, many genetic and epigenetic changes implicated in aging and longevity are associated with aging in model organisms. Even though their role and action in human aging are uncertain, many of these changes are also associated with non-communicable diseases. Recent discoveries showing that mechanisms involved in cancer are strongly associated with the aging process have led to multiple proposals to prevent cancer and other age-related diseases using drugs that increase lifespan in model organisms. AI and machine learning in the course are an inseparable aspect of modern medicine, especially tackling the prevention of global burden diseases. AI is expected to have a major impact in healthcare, where it can be used for the development of effective personalized medicine based on the interpretation of large medical databases gathered over the years by companies and healthcare providers.

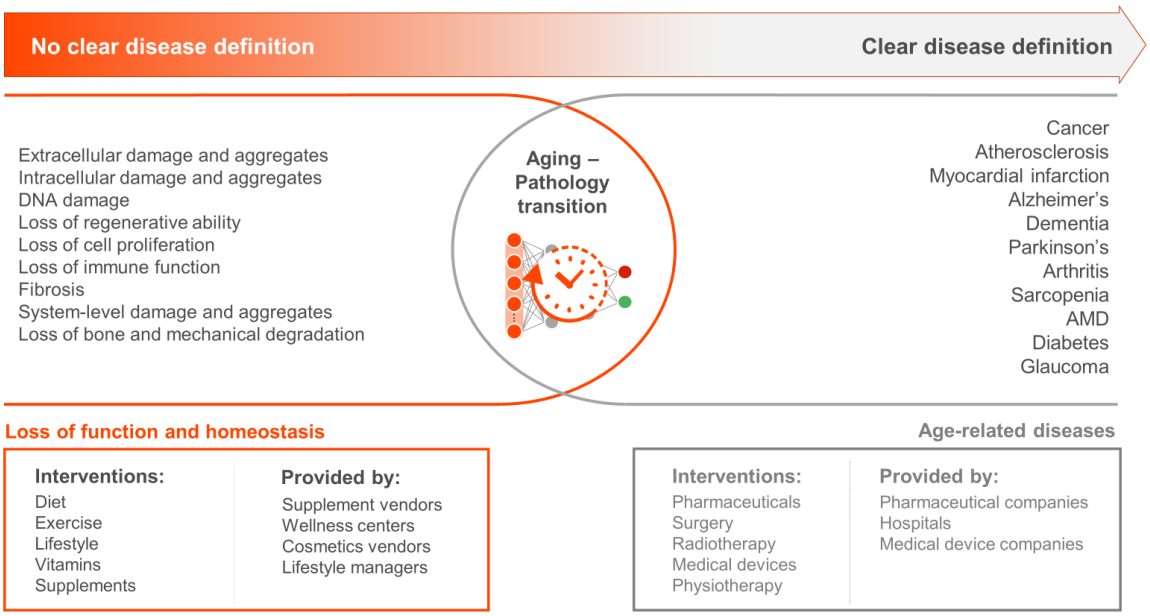


Figure 3. Cellular processes with no clear disease definition contribute to aging and pathology over time and are affected by environmental factors such as diet. These can then lead to age related disease.

Healthy vs. wealthy longevity - longevity medicine and public health

The debate around longevity medicine allowed a valid, but easily refutable argument that it might lead to an increase in health inequity. There are abundant reasons to counter-resonate. Firstly, novel technologies and interventions are developing rapidly in a competitive ecosystem. We have seen a decline in e.g. genetic testing pricing or CT/MRI imaging. With this trend, the longevity medicine might actually be one of the most affordable domains in terms of diagnostics and follow up, as well as regarding geroprotectve supplements or even specific invasive interventions, such as plasmapheresis. Secondly, most of the hallmarks of aging can be co-influenced by lifestyle modifications (exercise, nutrition, supplements, caloric restriction, intermittent fasting, cognitive activities etc.), led by inexpensive app-based solutions, such as DACs, CGM. Such management towards risk prevention and short-term improvement of performance does not require strong financial inputs and is accessible to the majority of digitalized populations. An accompanying longevity physician navigating the measurements and interventions is an optimal way too fully extrapolate and exploit these tools, since they are able to customize the interventions and interpret the measurements for an individual, minding the biovariability, comorbidities, chronological age, and – importantly – personal goals and preferences (which determine the compliance). As mentioned previously, these experts are still underrepresented and implies a speedy advent of appropriate education. Thirdly, healthcare institutions across virtually all countries globally face overcrowding and limited resources due to high healthcare costs, which in return aggravate the health inequality and inequity. Most cost burden derives from age-related and chronic diseases. Mitigating those is now a priority in the medical and scientific field, as to circumvent a clash of health systems challenged with the spiralling chronic multimorbidities. Another example is the simple illustration of cost- and time-efficacy of AI-based drug discovery and repurposing, which allows to save millions of USD and several years on identification and testing of new compounds. At the moment, the sobering facts are: 90% of all drug trials fail, very few that do

succeed take an average of 10 years to reach the market and cost range from $2.5 to $12 billion. In addition, in-computero clinical trials simulations are promising to bring more equity: algorithms can be trained and re-trained to include features that are largely ignored, such as the aspect of biological sex and gender, elderly, multimorbid patients, ethnical minorities, and importantly – the age etc.

Surely, as for any emerging field, also precision medicine will face barriers related to socio-economic inequities and demand solutions towards financial viability, especially in systems based on solidarity. Targeting and empowering coordinated discussions of multidisciplinary stakeholder by continuous updates on the current state of science by KOLs in a transparent manner is a crucial approach towards a successful democratization of longevity medicine for all.

Since healthy longevity medicine is precision medicine driven by aging biomarkers and is not seeking a bare extension of life, but an extension of a healthy, productive lifespan, the field can notably contribute to improve the economy of healthcare and as a whole.

AI applications in medicine – the fundament of precision medicine

Tangible AI applications in medicine are rapidly increasing in number, complexity and accuracy, even though overall, they are still in very preliminary stages. The vast variety of areas and problems that securely designed AI systems in medicine can tackle in practice bears major opportunities towards improvement of the healthcare as entity. At the entrance of the new decade (2020), most AI appliances are targeted at assisting physicians in their diagnoses and treatment decisions. The ultimate goal for the reactive medicine is to achieve an individualized prediction which treatment will work for which patient and how well in order to avoid chronification, physical and emotional burden and costs. There are several overarching AI-appliances that trespass current healthcare. Firstly, the basic prerequisite for AI applications – the data, are now mostly collected in a planned electronic manner. Secondly, AI allowed telemedicine to be established and flourish speedily. Public health impacts are indisputable alone through the provision of medical care in rural regions with a low density of specialists (thus: faster diagnosis, better prognosis, less morbidity and mortality, less costs, less burden on the patients and caregivers etc.).

AI-based diagnostic systems are able to detect features quickly, quantitatively, objectively, and reproducibly, and thus classify conspicuous skin lesions with high accuracy. Machines learning supports diagnostics in numerous specialties, e.g. oncology (lung cancer detection), neurology (CT-assisted stroke detection), ophthalmology (early detection of maculo- and retinopathies), cardiology (risk assessment of myocardial events, sudden cardiac death based on ECG and cardiac MRI), dermatology (detection and classification of dermal lesions based on a body scan or even images) etc. The latter exemplifies the abundant potential of AI-applications in patient care, e.g. remote care, patient engagement, auto-monitoring. Even though the dermatologic smartphone apps for self-examination by patients are still under a strict scrutiny and depend on compliance, pre-identification of suspicious skin lesions by the AI algorithm and a following analysis by specialists, improves the early detection of e.g. melanoma AppDoc - Online Dermatologist" and the "Derma-App" are prominent examples in Germany. More studies are needed to confirm the outperformance of AI over dermatologists, based on verified benchmarks. A close follow-up of conspicuous lesions in high-risk patients is further enabled with digital dermoscopy, a 3-D whole-body photography.

AI can further facilitate and optimize medical processes, such as division and delivery of blood products – a problem that is virtually universal and leads to massive losses in resources (both time and costs). "AutoPiLoT" AI-system and app for example approaches this issue by evaluate past data and thus be able to make predictions amount and timing of blood units needed in a specific hospital, as well as to recognize patterns that can further be readjusted individually. The app has now also implemented blood donation, capturing the donor data and thus simplifying the matches.

AI can also simplify the diagnostics of complex diseases, respectively complex diagnostic tools, such as MRI in multiple sclerosis (MS). AI algorithms, encapsulated in a user-friendly app, will allow non-experts (such as GP) to accompany the patient, interpret complex images over time, gain experience.

Tailoring an optimal treatment for a patient involves all aspects of the clinical picture is considered, but optimally also other information about the patient's biological features (e.g. genetic data), health data and examination results. Data from a large number of patients must be analyzed and intelligently linked to predict the course of the disease and the optimal therapy for a specific group of patients sharing similar features. AI is incumbent in order to conduct these steps and thus enable what is defined as precision medicine.

Conclusion and future perspectives

Longevity medicine is a groundbreaking dynamic field, emerging as one of the most essential medical disciplines combining the most advanced and complex diagnostic and interventional approaches. As an AI-driven precision medicine, longevity medicine harnesses innovative and state-of-the art technologies and science to exploit the potential of the human genome, deep quantitative phenotyping, -omics (e.g., epigenomics, metabolomics, proteomics etc.), microbiome, radiogenomic precision imaging etc. With the help of continued data collection, the development of new features, improvements in ways of interpretation and further optimizations in the implementation of an individual patient protocol, longevity medicine will be self-perpetuating. The longitudinal approach enables a trifold dynamic, interrelated longevity practice: data mining, patient compliance and physicians' lead. This shifts reactive medicine with limited human data analysis capacity towards longevity doctors that can collect and apply gigabytes of patients' data towards identification, mitigation and elimination of actionable diseases, preferably years and decades ahead.

Part IV. Cardiooncology and geroncology

Chapter 9. Geroncology – definition and novel approaches, personalized precision cardio oncology

"Hypoxia in breast cancer – scientific translation to therapeutic and diagnostic clinical applications". Bischof E, et al. Front Oncol. 2021 Mar 11;11:652266

Abstract

Breast cancer has been the leading cause of female cancer deaths for decades. Intratumoral hypoxia, mainly caused by structural and functional abnormalities in microvasculature, is often associated with a more aggressive phenotype, increased risk of metastasis and resistance to anti-malignancy treatments. The response of cancer cells to hypoxia is ascribed to hypoxia-inducible factors (HIFs) that activate the transcription of a large battery of genes encoding proteins promoting primary tumor vascularization and growth, stromal cell recruitment, extracellular matrix remodeling, cell motility, local tissue invasion, metastasis and maintenance of the cancer stem cell properties. In this review, we summarized the role of hypoxia specifically in breast cancer, discuss the prognostic and predictive value of hypoxia factors, potential links of hypoxia and endocrine resistance, cancer hypoxia measurements, further involved mechanisms, clinical application of hypoxia-related treatments and open questions.

Introduction

Breast cancer has been the most commonly diagnosed cancer and the leading cause of cancer deaths among women, accounting for approximately 630,000 deaths in 2018. It is a highly heterogeneous disease and clinic-pathological factors as well as multi-genomic assays allow for a sub-classification into several types and diverse subtypes, with different biological features and prognoses as well as different response to treatments. Breast cancer mortality has decreased since the early 1990s (absolute reduction of 39% from 1989 to 2015) due to a combination of improved prevention, screening and earlier detection/diagnosis, lifestyle changes and awareness, as well as significant improvements in anti-cancer therapies. Despite these advances, worldwide every minute a woman dies from breast cancer. While mortality has been decreasing the incidence of breast cancer has been increasing. In the US, every 2 minutes and in the EU, 8 women are newly diagnosed every hour.China is another giant, modern society, where the burden of cancer is reaching epidemic proportions. In 2015, China National Cancer Center reported 12,000 per day (4.3 million) newly diagnosed cancer cases, accounting for a quarter of the global prevalence, out of which 15% are attributed to breast cancer (in women).

Hypoxia is a characteristic feature of cancer. Tissue microenvironment influences tumorigenesis and tumour progression. Most solid tumor types have been shown to exhibit regions of hypoxia. The presence of a hypoxic microenvironment is a recognized event in mutagenesis and cancer development. At the same time, cancer per se also induce hypoxia secondary to inflammation.

At the present time, various studies demonstrated a correlation between hypoxia and carcinogenesis, metastasis, treatment failure and patient mortality[8-10]. About 25-40% of

invasive breast cancers exhibit hypoxic regions. Local hypoxia within the tumour and surrounding microenvironment is mainly a result of anabnormal anatomy of blood vessels, excessive angiogenesis leading to local obstructions or compressions and disturbed microcirculation. Generation of a hypoxic environment and the activation of its main effector, the hypoxia-inducible factor (HIF)-1, are even more common features of advanced malignancy.

Hypoxia may retain cancer stem cells in their undifferentiated state, permitting solely cancer cells to differentiate and uninterruptedly accumulate genetic and epigenetic changes over a period of time. Intra-tumoralhypoxia increases the number of breast cancer stem cells (BCSCs), which are essential for disease progression and recurrence. Hypoxic breast cancer (and other) tumorsare associated with a more aggressive phenotype, increased risk of metastasis and resistance to anti-cancer treatments.

In this review, we address the role of hypoxia in breast cancer, discuss unanswered questions and potential hypoxia-related treatments, in the context of relevant published literature.

Hypoxia-inducible Factors (HIFs) and breast cancer

The response of cancer cells to hypoxia is principally ascribed to HIFs, whichare composed of aHIF-α(HIF-1α, HIF-2α, or HIF-3α) and a HIF-1ß subunit. These HIFs are responsible for the majorityof the hypoxia-induced changes in gene expression. HIF-1α-mediated mechanisms favor tumor growth andmalignant progression, up- and down-regulation of genes, as well as pathologicmodifications of the genome, whereas HIF-2α stimulates some, but not all genes activated by HIF-1α. HIF-1αresponds in transient manner to severe hypoxia with rapid stabilization and activation of target genes, whereas HIF-2α responds to moderate levels of hypoxia and accumulates over time.

Under normoxic conditions, HIF1αis degraded by the proteasome, while under hypoxia, it translocates to the nucleus and forms a heterodimer with HIF-1β, which triggers the hypoxic response- a coordinated gene expression program. The hypoxic response triggers a decrease in cellular metabolism, thus inactivating the mammalian target of rapamycin (mTOR) pathway.

In addition, HIFs activate the transcription of a large battery of genes encoding proteins that promote primary tumor vascularization and growth, stromal cell recruitment, extracellular matrix remodeling, cell motility, local tissue invasion, metastasis, and maintenance of the cancer stem cell properties. Overexpression of HIF in breast cancer was often proposed as an unfavorable feature.

Hypoxia and the expression of hypoxia-mediated proteins, such as HIF-lα and VEGF, have been suggested to be negative prognostic and predictive factors, owing to its multiple contributions to chemo- and radioresistance, angiogenesis, invasiveness, metastasis, resistance to cell death, altered metabolism and genomic instability. As reported in a variety of studies, HIF-1αoverexpression is significantly correlated to adverse outcomes and a poorer survival in breast cancer patients. Increased concentrations of HIF-1αhave also been independently associated with a worse outcome, as demonstrated by immunohistochemistry in subsets of biopsies analyzed from both lymph node-negative and lymph node-positive breast cancer patients. Moreover, higher levels of both HIF-1αor HIF-2α in breast cancer biopsies are associated with metastasis to regional lymph nodes and distant organs, primary mammary tumor growth, as well as with an increased patient mortality. The proposed mechanisms

involve tumor-infiltrating cells (TICs). HIF-1α expression rises alongside tumor grading, being higher in less differentiated than in well-differentiated lesions. Finally, HIF-1α was proposed as a prognostic marker for an unfavorable outcome in those with T1/T2 tumors and positive axillary lymph nodes.

HIF-1β has also been reported to correlate with more aggressive cancer characteristics and poor survival, but the difference was not statistically significant in multivariate analysis.

Hypoxia in breast cancer development and progression

Hypoxia plays an important role in tumor progression and development. Related processes include the meditation of angiogenesis, apoptosis, the glycolytic shift and the recruitment of tumor-associated macrophages.

Angiogenic growth factors and their receptors are significantly up regulated in response to hypoxia, which causes vascular effects including endothelial cell migration with increased vascular permeability and promotes tumor angiogenesis. During these phases of transformations and growth, as the vessels are loosening their hierarchy and become arbitrarily arranged, cancer and stromal cells have a restricted access to nutrients and oxygen. Oxygen partial pressure in the tumor is significantly lower than in the healthy tissue at the tumor margins. The tumor cells in the vicinity of perfused vessels obviously still benefit from their oxygen supply, while cells at a greater distance are particularly hypoxic. This exacerbates even more in anemic cancer patients. Moreover, when a region of hypoxia is encountered, tumor-associated macrophages (TAMs) are induced to accumulate and exhibit a tumorigenic phenotype. TAMs can also secrete angiogenic growth factors and are associated with angiogenesis and poor prognosis in invasive breast cancer.

Coordinated regulation of a number of pro- and anti-apoptotic pathways by both HIF-dependent and HIF-independent mechanisms governs susceptibility to hypoxia-induced apoptosis in a cell-type-specific manner.

Hypoxia inhibited the pro-apoptosis effects of serum deprivation, reduced thebax/bcl-2 ratio, decreased cytochrome c release and caspase 3 activity via induction of vascular endothelial growth factor (VEGF). Also, hypoxia selects for $p53^{mut}$ cells with elevated levels of apoptosis inhibitor bcl-2. The reduced ratio of p53/bcl-2 acts increases mutation rates within a clone population, promoting the oncogenesis of breast cancer. Normally, such hypoxic state, if persistent, causes apoptosis of healthy cells, while some tumor cells stop dividing, but continue to exist and others (with a certain genetic predisposition) succeed in surviving and continue to be destructive. Out of many tumor cell populations, mechanism of selection will lead to a preference of those capable to assimilate and thrive under hypoxic conditions. These are particularly aggressive as they usually become apoptosis-resistant and thus the responsiveness to radiation or chemotherapy is reduced.

In addition, hypoxia maximizes the efficiency of the glycolytic shift via changes in the expression of glycolytic enzymes and glucose transporter genes. Both the maximal glucose uptake and high efficiency of glucose utilization lay the basis for glycolytic respiration, which enables tumor cells to grow and proliferate under such conditions.

Extracellular matrix (ECM) is a network of proteins and proteoglycans, which supports diverse cellular functions. Apart from the direct increase on endothelial cells (ECs) via the expression

of matrix metalloproteinase (MMPs), ECM is also involved during hypoxia-driven angiogenesis. Numerous studies highlighted that hypoxia regulates the expression and stability of ECM proteins (collagen I and IV and laminin) in cancer cells. ECM deposited from co-cultures of Neonatal Fibroblasts (NuFF) with breast cancer cells supported 3-dimensional vascular morphogenesis. Hypoxic fibers occupied a greater percent area and possessed larger diameter fibers than those deposited by co-cultures innormoxic conditions. It has been reported that HIF-1α is related to the changes in fiber organization, given that fiber alignment was abrogated in hypoxia-treated fibroblasts when HIF-1αwas knocked down. Overall, a disturbed and overloading structure results. This activates angiogenic responses by promoting up-regulated expression of vascular pro-angiogenic factors VEGFAand Ang1, proteolytic enzymes MT1-MMP and MMP1, while leading to a down-regulation of the vascular destabilizing factor, thus altering EC responses. In sum, not only the architecture, content and order of the EC are modified, but the functional aspects as well.

Hypoxia prognostic factors in breast cancer

Several recent studies have shown independent prognostic significance of a number of hypoxia related factors, such as PGC1, transcription intermediary factor 1γ (Tif1γ) or transforming growth factor -β (TGF-β), similarly to HIF1α, where this has been confirmed before.

TGFβ has been shown to have both tumor suppressive (early stages) and oncogenic (later stages, pro-metastatic and pro-EMT) effects. Especially the isoform TGFβ1 is an inhibitor of mammary gland epithelial cell proliferation and plays an important role in breast carcinogenesis.

TIF1γ contributes to breast cancer by controlling TGF-β/Smad signaling, leading to a TGF-β-induced EMT. A link was reported between TIF1γ and HIF1α in TNBC. In a study in press, we were able to show that the levels of Tif1γ were significantly lower in patients with breast cancer than in healthy controls. The average concentration of Tif1γ-discriminated between Tif1γ-positive and Tif1γ-negative patients. The latter group had a significantly worse OS (P = 0.0174); this was confirmed in the multivariate analysis. Tif1γ plasma level seems to be thus an independent prognostic factor for patients with breast cancer. This supports the potential of using measurements of Tif1γ plasma level to guide breast cancer therapy and monitoring.

Other proteins involved in cell homeostasis might become additional biomarkers in the early detection/diagnosis and monitoring of breast cancer if their apoptotic features react to the influence of aerobic versus anaerobic microenvironment. Further studies are required to identify and validate new easily detectable, non-invasive biomarkers with prognostic power – studies of some such biomarkers are already ongoing, e.g.PGC1α, Tif1γetc.).

Increased levels of PGC1α were, similarly toHIF-1α, associated with more aggressive tumors -e.g. histologically higher grade and higher stage - and were therefore proposed as a prognostic marker for unfavorable outcomes, especially in positive axillary lymph nodes tumors. Recently, PGC1α was confirmed to be an independent prognostic marker, where over-expression correlates with poorer outcome in an unselected (all stages) breast cancer population[40].
All these markers (PGC1α, HIF-1α and Tif1γ) can be measured easily from patients' plasma, which allows a simple, cost- and time-effective method for an improved clinical decision-making regarding treatment or even an early diagnosis for patients.

Hypoxia and breast cancer metastases

Despite the rapid progress in breast cancer treatment, the development of metastases remains the primary reason for breast cancer mortality. It is a complex process, which until now is known as a series of steps: epithelial-mesenchymal transition (EMT), local tissue invasion and intravasation, extravasation and metastatic niche formation. As mentioned above, hypoxia contributes to cell transformations, so that they undergo fundamental functional and structural changes. In this manner, a number of those cells, who were previously sedentary cancer cells, acquire properties that are essential for mobilization, conspicuously due to genetic modifications in p53 (tumor suppressor) and modifications on chromosome level (e.g. in Chromosome 1).

EMT is characterized by cellular and molecular changes that include loss of cell-to-cell adhesions. Thus, phenotypically, the migratory cells possess little or no adhesion molecules, while they develop a battery of lytic enzymes to invade lymphatic and blood vessels. HIF-1 activates EMT through regulating associated signaling pathways, modulating EMT-associated inflammatory cytokines, as well as interfering in other pathways, such as epigenetics (here, concrete data are still missing). Transcription factors like E-cadherin, SNAIL (zinc finger protein snail), ZEB1 (zinc finger E-box-binding homeobox 1) and TWIST are also involved in theHIF-1 induced EMT.

MMPs degrade many of the components of the ECM, which enables cancer cells to invade the surrounding tissues and intravasation. Hypoxia and HIF-1 up-regulate the expression levels and/or MMP-2 and MMP-9, which are positively correlated with a higher incidence of metastases and with a poor prognosis. HIF-1αplays a critical role in collagenogenesis by up-regulating the expression levels of pro-collagen prolyl (P4HA1 andP4HA2) and lysyl hydroxylases (PLOD2), which are reported to be crucial for breast cancer metastasis.

Hypoxic breast cancer cells produce multiple members of the lysyl oxidase (LOX) family, including LOX, LOXL2, and LOXL4, in a HIF-1-dependent manner. LOX remodel ECM both in the primary and the distant site, which provokes metastatic niche formation. Furthermore, HIF-1 induces miR-210-expression, a non-coding RNA, which contributes to tumor proliferation and forming of metastasis, alongside of other non-coding RNAs (miRNAs and lncRNAs).

Hypoxia and breast cancer stem cells

Breast Cancer Stem Cells (BCSCs) are characterized by an unlimited self-renewal differentiation potential, performance of symmetrical and asymmetric cell divisions, as well as regeneration[68]. In mesenchymal stem cells (MSC), it was shown that most of them are exposed to a lower oxygen concentration in vivo, e.g. by about 7% in the medulla or adipose tissue. Ex vivo, culturing of MSC under hypoxic oxygen concentrations resulted in a higher growth rate, glucose consumption and longer life at a constant level of the stem cell functionality.

The metastasis-promoting effects of HIF-1 help to maintain an expanding renewing population of BCSCs ready to be distributed much like seeds or pollen blowing in the wind. Increased expression of HIF-1αand HIF-2α in BCSCs lead to increased expression of pluripotency factors such as NANOG, OCT4, SOX2, and KLF4 in response to intratumoral hypoxia. HIFs also mediate complex and bidirectional paracrine signaling between breast

cancer cells (BCC) and MSC that stimulate breast metastasis. Interactions between BCC and MSC are supposed lymediated by CXCL10→CXCR3, CCL5→CCR5, andPGF→VEGFR1 signaling in a HIF-dependent manner. Further research is needed to explore these interdependencies more fully.

A link between obesity and breast cancer via hypoxia?

Under hypoxic conditions in the breast cancer tissue, the adenosine receptor 2B (A2BR) is overexpressed. When activated (among others also through HIF1 factors), A2BR leads to the activation of protein kinase C-δ, transcription factor STAT3, interleukinesIL6 and NANOG. The two latter mediators are essential for BCSC. Experiments in vitro showed that both a drug-related or genetic inhibition of A2BR expression or functionality lead to a decrease in BCSC enrichment, significantly reducing the tumor initiation and metastasis. These findings are fundamental to understand the known link between obesity and more aggressive breast cancer characteristics, as well as the higher risk of developing postmenopausal breast cancer.

Hypoxia and treatment resistance

Hypoxia is known to directly or indirectly confer resistance to irradiation, some chemotherapeutic drugs, and endocrine therapy. Hypoxic tumors are less responsive to radiation therapy, mainly because the lack of oxygen causes DNA damage. Moreover, the responsiveness of malignancy to chemotherapeutic agents is modulated by reducing the susceptibility to DNA damage, inducing cell cycle arrest and limiting drug delivery underpoor perfusion. The activation of ROS-shielding pathways and overexpression of anti-apoptosis genes mediated by hypoxia contribute to taxane resistance. As for ER-positive breast cancer patients, hypoxia has been shown to down-regulate ERα in several breast cancer cell lines and to influence the responsiveness to tamoxifen. SNAT2, an amino acid transporter, was regulated by both ERα and HIF-1α (predominantly), leading to endocrine resistance under hypoxia.

While suppressing VEGF pathway initially decreases tumor progression rate and vasculature density, the activation of interrelated pathways and signaling molecules following VEGF blockade compensates the insufficiency of VEGF and the initially blocked angiogenesis, explaining part of the failure observed with bevacizumab monotherapy.

Future Measuring hypoxia in the breast

An innovative method was tested in Austria: the possibility of hypoxia measurement in the breast using magnetic resonance imaging (MRI). This opens up new avenues of research into hypoxia although at this stage MRI has clearly not been established in the clinical approach. Besides measuring oxygen content, the MRI can also assess neovascularization in breast tumors. A significant benefit of this approach would be lower costs and greater availability compared to PET or near-infrared spectroscopy. Advanced quantitative blood oxygenation level dependent (qBOLD) imaging can directly quantify the tissue oxygen tension, while vascular architectural mapping (VAM) measures the microvascular vessel diameter and architecture. This approach looks potentially promising but further research is clearly required to validate any clinical utility.

Gene expression and hypoxia in breast cancer

The adaption to hypoxia is governed by multiple transcriptional and post-transcriptional changes in gene expression. Up to 1.5% of the human genome is estimated to be transcriptionally responsive to hypoxia. Recent years brought insights into various additional genes and pathways that have been identified as being responsive to hypoxia and which might serve as prognostic or predictive markers, and even as novel therapeutic targets. Clustering genes are chosen for their expression pattern. Since increased activity of the HIF-1a pathway is related to a more profound intratumoral hypoxia in basal-like breast tumors compared with other subtypes, gene signatures might guide treatment decisions for potential application of anti-hypoxic drugs in the future.

Gene signatures reflect hypoxic response at a transcriptional level, whereas microRNAs regulate it at a post-transcriptional level. Comparative analysis of hypoxia-regulated miRNAs by gene expression profiles might add additional information to target-prediction algorithms. Despite rapid development, this area still needs further clinical validations.

Anti-hypoxic treatment

HIF-1α promotes primary breast cancer growth, vascularization and metastases to axillary lymph nodes and distant organs. Increased HIF-1 expression shows strong correlations with poor prognostic outcomes and low survival rates of breast cancer patients. Therefore, targeting the HIF pathway might provide an attractive strategy to treat hypoxic tumors. Agents that inhibit HIF-1α protein accumulation and demonstrate anti-tumor effects include the topoisomerase I inhibitor, topotecan, as well as the cardiac glycoside digoxin.

Since HIF-1α can be induced by hypoxia-independent signaling pathways, such as motor, ERBB2 (HER2) and MAP kinase, the therapeutic benefits of targeting these pathways may also be partially explained by a decrease in HIF-1α levels. Especially triple-negative breast cancers (TNBCs) have a high HIF transcriptional activity and respond poorly to currently available therapies. Therefore, HIF inhibitors may be particularly useful in the treatment of TNBCs. Preclinical studies suggest that the combination of cytotoxic chemotherapy with drugs that inhibit hypoxia-inducible factors are very promising in this group of patients. HIF-1 inhibitors, such as digoxin and acriflavine, showed convincing potential therapeutic effects by decreasing primary tumor growth, vascularization, invasion and metastasis in breast cancer animal models. Adding digoxin to paclitaxel or gemcitabine leads to tumor regression in TNBC by blocking HIF-dependent transcriptional responses that promote the resistance of CSCs to chemotherapy.

Another aspect might be hypoxia-based treatments, where a synergetic effect with drugs that cause treatment-induced hypoxia, e.g. bevacizumab, is applied. Despite compelling evidence-linking hypoxia with treatment resistance and adverse prognosis, the activity of hypoxia-activated drugs also depends on the coincidence of tumor hypoxia, expression of specific prodrug-activating reductases and intrinsic sensitivity of malignant clones to the cytotoxic effector. Hypoxia-based drugs have been tested in clinical trials to further validate their efficacy in cancer treatment. However, the failure of two major clinical trial efforts (tirapazamine and evofosfamide) calls for further research. Hypoxia itself is highly variable between and within individual tumors and is not consistent among all breast cancer subtypes.
In the era of personalized precision medicine, clinical trials are warranted to determine whether anti-hypoxia drugs may increase the survival of breast cancer patients alone or in

combination with current therapeutic regimens. It is pivotal to explore the decisive influence of hypoxia on the course of breast cancer in order to gain a deeper understanding of individual disease trajectories and to better forecast them. This knowledge can then be used in the future to develop and implement adequate therapy for each individual patient.

Chapter 10. PGC-1α as a novel predictive and prognostic biomarker

"PGC-1α as a novel predictive and prognostic biomarker in breast cancer." Bischof et al. in preparation for Journal of Oncology

Background: Male breast cancer(mBC) has unique clinicopathologic and prognostic features. We previously identified PGC-1α as a prognostic marker for female breast cancer (fBC). Thus, our goal was to assess PGC-1α in mBC as a marker of prognosis and an indicator for early, subclinical metastasis, as well as to reconfirm our findings in female breast cancer.

Materials and Methods: 700 female and 23 male breast cancer patients were included. A clinicopathologic database including long-term clinical follow-up was created. PGC-1α levels were quantified by ELISA from plasma samples collected to therapy-naïve patients. Differences in baseline PGC-1α plasma levels and clinicopathologic features between males and females were calculated. Propensity Score Matching method (Match Ratio 1:4, Caliper=0.1) was applied to eliminate clinicopathological mixed bias in the two groups. Disease-free survival (DFS) curves were estimated and analyzed by the log-rank test.

Results: The PGC-1α plasma level was higher in fBC than in mBC (248.86 ng/dl: 224.96 ng/dl, p=0.040), where the female and male samples did not differ in clinicopathological features. A statistically significant association was found between high level PGC-1α and higher risk of recurrence. Significant survival priority of patients with low PGC-1α plasma levels was also found, whereas patients with high PGC-1α plasma levels had an almost significantly poorer DFS.

Conclusion: PGC-1α appears to be an independent marker of poor prognosis and more aggressive cancer characteristics. And may represent a non-invasive, innovative option to identify high-risk individuals, predict outcome, monitor treatment and screen for disease recurrence in mBC patients.

Background

Worldwide, male breast cancer (mBC) accounts for around 1% of all breast cancer cases, but the incidence has increased over the past 25 years. Although breast carcinomas in both genders share certain characteristics, male breast cancer appear to have some pathological and radiological differences from female breast cancer (fBC) and other notable discrepancies are being investigated, e.g. via genetic landscapes. In comparison to female breast cancer, the knowledge base on male breast cancer. A recent study reported that, despite similarities between male and female BC, males less frequently harbored *PIK3CA* and *TP53* mutations and losses of 16q, suggesting that at least a subset might be driven by a different repertoire of somatic aberrations.

In contrast to females, male breast cancer typically peaks at age 71 years, diagnosis is often late, with more than 40% of individuals presenting at stage III or IV disease. Luminal A subtype seems to be most frequent. Familial cases usually have BRCA2 rather than BRCA1 mutations, but the genetic identification of BRCA mutations is not routinely conducted, so that the positive mutations were not proven predictive as of yet. Hyperoestrogenisation resulting from Klinefelter's, gonadal dysfunction, obesity, or excess alcohol, all increase risk, as does exposure to radiation (Table 1). Advanced stage-related tumor characteristics (e.g. tumor size >2 cm and positive axillary lymph nodes) seem to be more common in men

compared with women, according to an analysis of the Surveillance, Epidemiology, and End Results (SEER) registry of almost 6000 males (and over 800 000 women). Data concerning hormone receptor status are largely inconsistent and contradictory, especially in terms of their prognostic value. Most studies reported a higher number of Her2 negative tumors among mBC, however the occurrence varies between 2% and over 20% (average for females. Some reports with smaller number of subjects reported an even higher percentage of Her 2 positive mBC (˜40%). Nevertheless, the correlation between hormone receptor status and survival (as contrasted to females are not mature to be demonstrated and thus only assumable. Especially since, despite the higher grades, the overexpression of aggressive tumor markers was less prominent (p53, Erb-B2) than the female counterparts. Furthermore, the evidence demonstrated that invasive male breast cancers derive from DCIS lesions, which share the ER/PR and Her2 status with their adjacent aggressive descendants. However, a subanalysis revealed that histologic grade was not correlated with clinical outcome, unlike what is observed in females. Prognostic and predictive markers are thus of uttermost importance and severely missing thus far.

Risk factor	Risk level
obesity	high
Klinefelter syndrome	high
gynecomastia	high
physical inactivity	moderate
exogenous hormone use	moderate
diabetes	moderate
prediagnostic endogenous estradiol	Moderate/low

Table 1. Common risk factors of male breast cancer

Equally challenging is the therapeutic management of mBC. Like diagnostics, treatment is based on the recommendations for breast cancer in women. While female approaches tend to be increasingly personalized and tend towards minimally invasive options, mastectomy with adjacent lymphadenectomy is still the primary operation due to a little breast tissue and mostly central tumor location. However, it can be assumed that breast-conserving surgery is not being considered in most cases. mBC is almost always estrogen-dependent, so most patients will be put on tamoxifen, since the mortality rate on aromatase inhibitors (AI) is significantly higher. Newer data however encourage the AI use in mBC, especially with addition of GnRH. Another example is the use of adjuvant chemotherapy. For females, despite reports where the disease-free survival and overall survival rates are not improved for small tumors, the majority still receive adjuvant chemotherapy. Its role in mBC is potentially equivocal and most males will be subjected to unnecessary adjuvant options. Markers that can be applied to guide the therapy choice are therefore indispensable and of a high clinical impact.

Recently, we detected PGC-1α in breast cancer tissue in our initial study and then found that PGC-1α plasma level was a predictive and prognostic marker in female breast cancer patients. PGC-1α is a key element of cellular adaptive mechanisms in tumor microenvironments, which - in the vast majority of solid neoplasms, including breast cancer - are hypoxic. Hypoxia is a recognized event in cancer development and has great mutagenic potential. Correspondingly, the cancer itself will cause hypoxia due to the inflammatory process, which will activate a series of cytokines and chemokines. Therefore, the hypoxic microenvironment is closely related to tumor growth, development, metastasis, treatment response and prognosis.

Our objective was thus to assess the utility of PGC-1α as a prognostic marker and an indicator for disease free survival (DFS) in mBC. We further investigated the relationship of PGC-1α to DFS in fBC and mBC patients. DFS and clinicopathologic differences of mBC and fBC cohorts were also described.

Material and methods

Study Population

In total, 700 fBC and 23 mBC de novo diagnosed, therapy-naïve patients as well as 30 males with lesions that were non-tumorous (non-mBC, controls) were followed for a median of 43 months (3 - 82) from September 2012 to December 2018. Histological diagnosis confirmation was mandatory. We excluded the patients with other diseases or malignancies before enrolling the patients. Associated clinicopathologic database in a long-term clinical follow up were established for all patients. All patients were Chinese males and females and observed until death or the end of the follow-up period.

Prior to plasma samples collection, no neoadjuvant treatments of any type were applied. The medical records were the main source of information for the DFS and other characteristics (sociodemographic) data. In case patients were lost to follow-up, we conducted the following steps in this order to secure information regarding survival status: 1) Contact the family doctor; if not successful: 2) Contact the family; if not successful: 3) Retrieve survival information from the Shanghai Cancer registry; if not successful: 4) Patient censored at the last date of follow-up.

Treatment protocols and follow-up were at the discretion of the provider and based on established guidelines at each follow-up, standard evaluation on clinical disease progression was performed.

Sample collection and process

5 ml of fasting blood sample were drawn from antecubital vein of male and female breast cancer patients in EDTA anticoagulative tubes. Centrifugation for ten minutes (1000 r/min) to separate plasma and stored in −80 °C for further analysis followed.

Quantification of PGC-1α level by ELISA

Frozen plasma samples were de-identified and assayed by ELISA. The concentration of PGC-1α in male and female breast cancer patients was be determined using the ELISA kits Human peroxisome proliferator activated receptor gamma coactivator 1α ELISA kit, DRE11477, Shanghai bioleaf biotech Co., Ltd. Shanghai, China, according to the manufacturer's instructions. The sample dilutor used for diluting plasma was analyzed in the kit as a blank control. The optical density (OD) of each well was then measured at a wavelength of 450 nm in Varioskan Flash Multimode reader (Thermo Scientific, Waltham, Massachusetts, US). The concentration of PGC-1α was calibrated with the PGC-1α standard curve. All assays were repeated as duplicates and the means of OD were used for further calculations.

Statistical analysis

Descriptive statistics was conducted by frequencies and percentage with categorical. Continuous variables were analyzed for normality allowing for parametric analysis. The differences in baseline PGC-1α plasma levels and clinicopathologic features in male and female breast cancer patients was detected by Student's t-test and Chi-Square tests. Propensity Score Matching method (PSM, Match Ratio 1:4, Caliper=0.1) was used to eliminate clinicopathological mixed bias in the two gender groups, thus to balance confounding factors. The clinical outcome endpoint was *disease*-free *survival* (DFS).

Disease-free survival was defined as the time from surgery to recurrence or death. Kaplan-Meier survival analyses was used to evaluate survival curves of each group and log-rank test was used to determine the DFS. Differences with a P-value <0.05 was considered to be statistically significant. Data processing and statistical analysis were performed using SPSS version 24.0 for windows with R-Essentials 24 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

In total, 723 (700 females and 23 males) breast cancer patients and 30 male non-cancerous tissues (controls) plasma samples were included in the analysis, Clinicopathological parameters are presented in Table 2. In unmatched sample, mBC patients were significantly older at diagnosis (average age fBC 53.7±11.6 years, mBC 62.9±9.2, p=0.001), presenting with a smaller tumor size (fBC 2.03cm vs. mBC 1.54cm, p=0.001) but higher lower histological grade (grade I+II fBC 81.3%, mBC 78.3%, p=0.02). The *rate of positive lymph nodes* and the hormone receptor (ER, PR) negativity were higher in fBC group, while mBC were more often Her2 negative (almost significant). *No* significant *differences* were observed in skin involvement rate, Ki67 expression and pathologic type between the two groups. After Propensity Score Matching method, *the* clinicopathologic characteristics at baseline were similar between the matched samples of 78 fBC and 22 mBC (Table 2).

Plasma levels of PGC-1α in male breast cancer patients and male controls

In male breast cancer patients' cohort, mean PGC-1α plasma levels was 224.96 ng/dl (95% CI 202.93-246.99). It was clearly higher than the level of PGC-1α plasma in male controls: 150.71ng/dl (95% CI 135.84-165.57, t=-5.959, p = 0.001) (Figure 1). These findings were *consistent with* the previously reported data in female cohort.

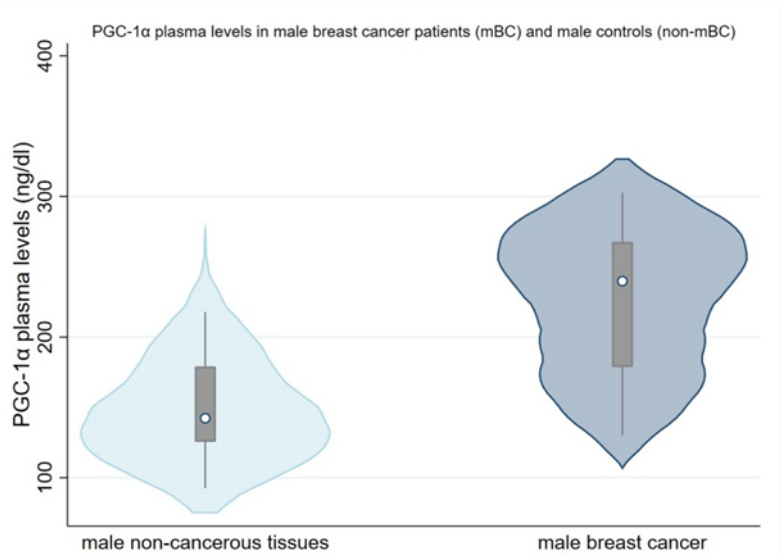


Figure 1. PGC-1α plasma levels in male breast cancer patients (mBC) and male controls (non-mBC).

Plasma levels of PGC-1α in overall and matched male and female breast cancer patients

In a total of 753 human plasma samples, PGC-1α plasma levels were measured by ELISA. The mean PGC-1α plasma level in fBC patients was 248.86 ng/dl (95% CI 242.92-254.81) and 224.96 ng/dl (95% CI 202.93-246.99, t'=2.163, p=0.040) in mBC patients (Figure 2). We further investigated the difference of PGC-1a expression in matched male and female breast cancer patients (after matching with Propensity Score Matching method (Match Ratio 1:4, Caliper=0.1, thus after eliminating confounding factors (clinicopathological features) between the two groups. In matched samples, no difference in clinicopathological features between male and female breast cancer patients was observed (Table 2). The mean PGC-1α plasma levels of 22 male breast cancer patients was 225.73 ng/dl (95% CI 202.52-248.76), thus non-significantly higher than that of 78 matched female breast cancer patients: 204.55 ng/dl (95% CI 194.31-214.79, t=1.860, p=0.066) (Figure 2).

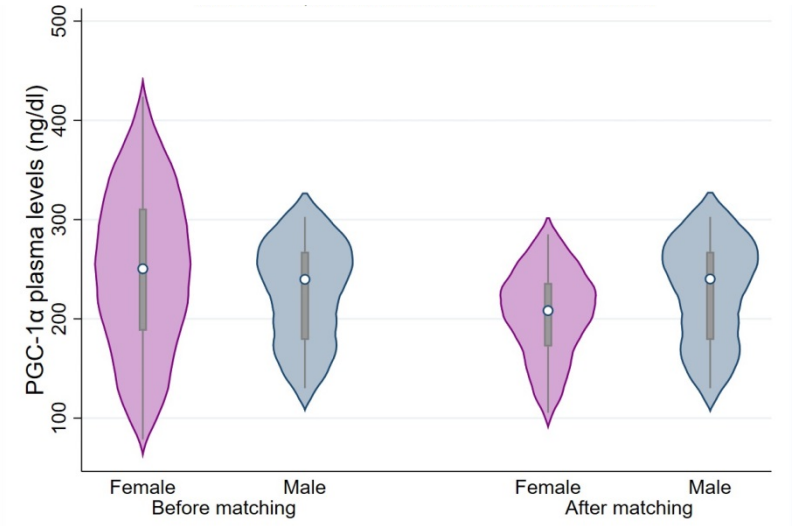


Figure 2. Mean PGC-1α plasma levels in unmatched and matched cohorts

Mean PGC-1α plasma levels in unmatched and matched cohorts was 248.86 ng/dl (95% CI 242.92-254.81) in female breast cancer cohort and 224.96 ng/dl (95% CI 202.93-246.99) in

male breast cancer patients (p=0.040). After Propensity Score Matching, the mean PGC-1α plasma level in 78 matched female breast cancer patients was 204.55 ng/dl (95% CI 194.31-214.79, and 225.73 ng/dl (95% CI 202.52-248.76; p=0.066, t test) in 22 matched male breast cancer patients.

	Original samples					Matched samples				
	Female (n=700)	Male (n=23)	Std. Mean Diff.	Statistical value	*P*	Female (n=78)	Male (n=22)	Std. Mean Diff.	Statistical value	*P*
Age (years)										
(Mean±SD)	53.7±11.6	62.9±9.2	0.996	t=-3.760	0.001	61.1±11.0	62.6±9.3	0.056	t=-0.592	0.555
Tumor Size(cm)										
(Mean±SD)	2.03±0.94	1.54±0.44	-1.099	t'=4.920	0.001	1.69±0.74	1.527±0.449	-0.270	t'=1.257	0.214
Skin involvement										
No	646(92.3%)	21(7.7%)		χ2=0.001	1.000	71(91%)	20(90.9%)		χ2=0.001	1.000
Yes	54(91.3%)	2(8.7%)	0.034			7(9%)	2(9.1%)	0.039		
LN metastasis										
No	454(64.9%)	19(35.1%)		χ2=3.102	0.078	62(79.5%)	18(81.8%)		χ2=0.001	1.000
Yes	246(82.6%)	4(17.4%)	-0.458			16(20.5%)	4(18.2%)	-0.088		
HR										
negative	188(26.9%)	2(8.7%)		χ2=3.791	0.052	5(6.4%)	2(9.1%)		χ2=0.001	1.000
positive	512(73.1%)	21(91.3%)	0.630			73(93.6%)	20(90.9%)	-0.105		
Her-2										
-~+	526(75.1%)	19(82.6%)		χ2=0.669	0.413	66(84.6%)	18(81.8%)		χ2=0.001	1.000
++~+++	174(24.9%)	4(17.4%)	-0.193			12(15.4%)	4(18.2%)	0.029		
Ki67										
<14%+	368(52.6%)	10(43.5%)		χ2=2.152	0.341	32(41%)	10(45.5%)		χ2=0.195	0.907
14-30%+	203(29%)	10(43.5%)	0.286			36(46.2%)	9(40.9%)	-0.157		

>30%+	129(18.4%)	3(13%)	-0.156			10(12.8%)	3(13.6%)	0.066		
Pathologic Type										
DCIS	26(3.7%)	2(8.7%)		Fisher	0.327	4(5.1%)	2(9.1%)		Fisher	0.610
IDC	655(93.6%)	21(91.3%)	-0.079			74(94.9%)	20(90.9%)	-0.158		
Other	19(1.7%)	0(0)				0	0			
Grade										
I	61(8.7%)	6(26.1%)		Fisher	0.019	17(21.8%)	5(22.7%)		Fisher	0.945
II	509(72.7%)	12(52.2%)	-0.402			45(57.7%)	12(54.5%)	0.001		
III	130(18.6%)	5(21.7%)	0.075			16(20.5%)	5(22.7%)	0.090		

Table 2 Clinicopathologic characteristics of male and female breast cancer patients in unmatched and matched samples.

Plasma level of PGC-1α and survival

In our study, 681 out of 723 patients completed the follow up of a median of 43 months (range of 3 to 82 months), including 658 female and 23 male patients, 16.6% (109/658) female patients and 21.7% (5/23, p-NS) male patients had local recurrence and/ or metastasis, 5.6% (38) died, 48.5% (33) due to breast cancer (4.7% (31) females and 8.7% (2) males). In original samples, *DFS* were not significantly different between male *and* female breast cancer patients. (p= 0.439, log-rank test, Figure 3A).

We performed a dichotomous division of unmatched patient samples by the median PGC-1α plasma level (246.81 ng/dl) in order to divide them into two groups: high- and low- PGC-1α level groups. Patients with high PGC- 1α plasma levels (>246.81 ng/dl) had a significantly *high risk of* tumor relapse and poor outcomes as compared to those with low PGC-1α plasma levels (p= 0.008, Figure 3B). When calculated only for the unmatched fBC patients, the results were similar: patients with high PGC- 1α plasma levels (>246.81 ng/dl) had significantly higher recurrence risk than fBC with low PGC-1α plasma levels (p= 0.016, Figure 3C).

In matched samples, 6.4% (5/78) of females and 22.7% (5/22) males had local *recurrence* or distant metastasis. The DFS of mBC patients was poorer than in fBC with statistically significant (p=0.033, Figure 3D), despite similar baseline clinicopathologic characteristics. We then performed the dichotomous division of matched patient samples into two groups (as above) by the corresponding median PGC-1α plasma level of 211.5 ng/dl. 47.4% (37/78) fBC and 59.1% (13/22) mBC patients showed high PGC-1α plasma levels (Pearson $\chi 2$=0.932, p = 0.334). Although analogous clinicopathological attributes, patients with high PGC-1α plasma levels had an almost significantly poorer DFS as compared to patients with low PGC-1α plasma levels (p = 0.057, Figure 3E).

4 out of 5 patients with breast cancer recurrence in the male group had high PCG-1α plasma levels. Similarly, high PCG-1α plasma levels were detected in 4 of 5 female patients with recurrences. As indicated by DFS curves constructed for the comparison of four different groups (based on survival results in correlation to gender and PGC-1α plasma levels), the prognosis in male patients with high PGC-1α plasma level was the worst one out of the four subgroups. The most significant difference was seen between fBC patients with low PGC-1α levels and mBC with high PGC-1α plasma levels (p=0.002, Figure 3F).

Discussion

Male breast cancer (mBC), steadily increasing in incidence over the past few decades, is a highly fatal disease. Recent data provide conflicting reports on female vs. male survival on outcomes in mBC compared to fBC are conflicting – while most of them suggest mBC to be associated with worse outcomes, some authors demonstrated comparable and even better survival rates in mBC. Nevertheless, it is undeniable that most male patients are diagnosed at an advanced stage, thus facing more aggressive therapies and a poorer prognosis. In addition, with the increase of the established risk factors of mBC, such as diabetes and obesity, oncologists will be challenged with this particular patients' population. Therefore, early predictive markers that would assist in clinical stratification between high risk and low risk patients are missing, especially since most established predictors of female breast cancer are not translatable into males. The recurrence rate of mBC patients in our study was higher and the DFS was significantly worse (p=0.033, Figure 3D), than among fBC despite similar rates of lymph node metastasis, skin involvement rate, Ki67 expression and hormone receptor (ER, PR) status, which is conform with the fact that these parameters cannot be used as prognostic and predictive indicators in mBC (unlike in fBC). PGC-1α is an easily detectable (plasma) protein that has demonstrated independent predictive and prognostic value in fBC. We thus aimed to study its potential use in mBC.

We prospectively investigated a cohort of female and male breast cancer patients. In unnmatched samples, the clinicopathological characteristics of both groups corresponded to the common distribution reported previously in descriptive retrospective analyses, with significantly older age and smaller but more aggressive (grade) tumors at diagnosis among males. We applied Propensity Score Matching method to control the confounders as to reduce bias and achieve more valid comparisons of female and male patient groups. The matched samples of 78 fBC and 22 mBC did not express differences in clinicopathologic characteristics.

Our findings showed that PGC-1α plasma level is a strong predictive and prognostic marker in male breast cancer patients. In addition, the mean PGC-1α plasma levels in the unmatched male sample (224.964 ng/dl) was significantly higher than the mean of PGC-1α plasma levels in male controls (non-malignant cohort of men, 154.045ng/dl(p = 0.001), confirming our previous observations in women and suggesting that PGC-1α plasma levels could play a diagnostic role in mBC patients.

We further investigated the difference of PGC-1a expression in male and female breast cancer patients. While different in unmatched samples (thus not representative), the mean PGC-1α plasma levels after matching were similar for both groups (matched mBC 225.7 ng/dl, matched fBC 204.553 ng/dl, p=0.0066, Figure 2). Furthermore, 8 out of 10 patients with breast cancer recurrence (4 of 5 in each group) had high PGC-1α plasma levels. We thus suggest that the higher PGC plasma levels in matched mBC patients correspond to more

aggressive cancer characteristics in that group, which further supports our hypothesis that PGC is a potential marker for patients' prognosis, risk stratification, therapeutic decision and monitoring (early detection of recurrences).

Both in unmatched and matched cohorts, patients with high PGC- 1α plasma levels had a higher risk of recurrence as compared to those with low PGC-1α plasma levels. In both cases the findings were statistically significant or almost significant (p= 0.008, p=0.057 Figure 3B, E). We assume that the samples size of mBC in the matched cohort was too small to reach the statistical significance. In addition, mBC patients with PGC-1α plasma levels had the worst prognosis out of all four analyzed subgroups (divided by gender and PGC-1α plasma levels, Figure 3F). Therefore, the clear trend, the strong statistical significance in the overall cohort and the nearly significant findings after matching allow to stand to reason that PGC- 1α plasma level is a solid marker for disease-free survival in mBC patients.

In summary, our research results indicate that PGC-1α may represent a non-invasive marker for screening mBC patients for disease recurrence, predicting the outcome, monitoring and adjusting treatment. In addition, inhibiting the PGC-1α pathway may be a promising anti-tumor treatment option.

Conclusion

PGC-1α appears to be an independent marker of poor prognosis and more aggressive cancer characteristics. And may represent a non-invasive, innovative option to identify high-risk individuals, predict outcome, monitor treatment and screen for disease recurrence. Furthermore, inhibition of PGC-1α pathways might be a promising antitumor management option. Our findings show that PGC-1α may represent a non-invasive marker to screen for disease recurrence, predict outcome, monitor and tailor treatment in mBC patients.

Chapter 11. Geroncology

"Management of Elderly and Old Cancer Patients-Geriatric Oncology." Bischof E, et al. healthbook TIMES Oncology Hematology 2021 Dec;10(4):28-33.

Abstract

Aging is a major risk factor for cancer development. Indeed, 60% of people with cancer in Switzerland and worldwide are older than 65 years. However, the indication for treatment choice should not be based on chronological age alone but instead driven by the patient's biological age, physiological and functional status, and the biological and morphogenetic tumor signature. This article provides an overview of the current challenges in treating elderly and old cancer patients, specifically the reasons for a less-than-optimal treatment and care and the added value of performing comprehensive geriatric assessments in this unique population.

Introduction

Cancer is the second leading cause of death in Switzerland. The incidence of cancer increases dramatically with advancing age, with a median age of onset around 65 years. By 2030, it is estimated that 70% of all new cancer cases will be diagnosed in patients 65 years and older. Unsurprisingly, cancer patients above 65 years have a 16-fold greater mortality risk than younger patients, with 70% of cancer-related deaths occurring in this age group. Early diagnosis and improved treatment options have led to a steady increase in the number of older adult cancer survivors in Switzerland.

Physiological changes that mostly occur with aging (e.g., decreased cardiovascular, respiratory and renal function) impair the tolerance of older patients to cancer therapy. Frailty, defined as an increased vulnerability to stressors due to a multisystem reduction in reserve capacity, is considered a distinct concept linked to higher chronological age and comorbidities. Meanwhile, the aging process is highly heterogeneous and influenced by multiple genetic, epigenetic and environmental factors, differing at all levels from the molecular to the system level. Therefore, frailty alone is not sufficient as a conceptual marker for cancer management but rather biological age. As long as the concepts of longevity medicine are maturing and artificial intelligence (AI)-based precision oncology is at the inceptive state of entering the clinical routine, the concept of frailty versus fitness, the so-called "Frailty syndrome", is gaining traction. Identifying frail cancer patients, i.e., those with high susceptibility, low functional reserve and unstable homeostasis, leads to a better estimation of individual response to therapies than looking at chronological age alone, therefore, careful consideration of each patient's status, including frailty in the treatment decision-making process is an integral part of oncological management. However, measuring and quantifying frailty in the real-world setting remains a challenge.

In addition to personal biological status, the optimal treatment in older cancer patients is challenged by the importance of maintaining quality of life (QoL). In routine clinical practice, the question often arises as to which treatment strategy is the right one for each patient, especially one that allows the patient to remain independent for as long as possible. Many older cancer patients are therefore at risk for both being undertreated and overtreated. Another challenge is that despite the increasing number of older people in the population and the relatively high frequency of cancer in this age group, patients 65 years of

age or older are significantly underrepresented in trials of cancer treatments. There is limited information concerning the efficacy, tolerability, and toxicity of cancer therapies in senior adults, although the literature indicates that this trend is slowly changing.

Clinical challenges and pitfalls in early palliative care of geriatric oncology patients

Palliative care is defined as an approach that improves the QoL of patients and their families facing the problem associated with life-threatening illness through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other physical, psychosocial and spiritual deficits. Evidence from the literature suggests that earlier integration of palliative care as part of a multidisciplinary team can facilitate optimum patient care. It should therefore be a part of the older cancer patient's care journey throughout the trajectory of their disease, with varying levels of multidisciplinary involvement as the disease progresses. However, providing appropriate care for older cancer patients is confounded by many factors, including multiple comorbidities and polypharmacy, frailty, dementia and cognitive problems, delirium, and functional impairment. In addition, approximately half of cancer patients will have a formal psychiatric disorder such as depression or anxiety. Although psychiatric illnesses are common, they are often neglected, which leads to reduced QoL, lower adherence to treatment, poorer cancer survival and increased treatment costs. In the field of oncology, the use of palliative care has increased significantly over the past decade. A US-based observational study found that patients who received palliative care experienced significantly less aggressive care, lower rates of hospitalization, increased use of hospice, and fewer invasive procedures near the end of life than patients that did not. Furthermore, negative public views and misconceptions about palliative care still exist within the community; for example, palliative care is often falsely equalized with end-of-life care and hospice care and thus death. This may partly be due to the fact that many elderly cancer patients prefer supportive symptom management and refuse further aggressive treatments such as chemotherapy routinely given to younger patients Another challenge is the lack of literature that specifically addresses geriatric palliative care across the domains of palliative care. Additional barriers to ensuring older cancer patients receive appropriate palliative care provision include the fact that some elderly patients lack capacity as a result of confusion and delirium, they may have limited social and family support, i.e., older patients tend to live further away from families and have fewer friends to support them, and have a lack of psychosocial support to combat loneliness and depression, especially since many older people tend to live alone.

Age-related factors influencing treatment of elderly cancer patients

Treatment decisions for older cancer patients must be made alongside other medical conditions such as cognitive impairment, depression, polypharmacy secondary to multiple comorbidities. Over the years, a lack of clinical data has led to a paucity of appropriate treatment guidelines and absence of a standard approach in this population. Since older adults with cancer represent a diverse cohort of patients with other comorbidities that may have an equal impact on survival and QoL as the diagnosis of malignancy itself, a competing risk approach design for clinical trials has been proposed.

Immune checkpoint inhibitors are a well-established treatment for all types of cancers and are generally better tolerated than cytotoxic chemotherapy. Treatment with anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) agents is, therefore, an attractive option for elderly patients with multiple comorbidities. Pooled data on elderly patients (aged

75 years and above) with PD-L1-positive advanced non-small-cell lung cancer (NSCLC) from three landmark cancer trials were recently analyzed. Results demonstrated that immunotherapy improved overall survival in these patients compared with chemotherapy, and results were comparable to the overall population results in the individual studies. Moreover, the older NCSLC patients treated with immunotherapy experienced fewer treatment-related adverse events (TRAEs) than those that received chemotherapy (grade 3−4 TRAEs: 24.2% versus 61.0%). Similarly, in a real-life study of the anti-PD-L1 inhibitor nivolumab, reported by Grossi et al. (2019), almost half of the NSCLC participants (N=1588) were elderly patients aged 70 or above (n=754). In a subgroup analysis, elderly patients receiving immunotherapy with nivolumab achieved outcomes similar to the overall study population, with an overall survival rate of 43% at 12 months.

It is widely known that an aging immune system (immunosenescence) may affect outcomes for cancer patients. The main hallmarks of immunosenescence include a reduced ability to respond to new antigens, the accumulation of memory T cells and the lingering level of low-grade inflammation. For example, in patients receiving immunotherapy for melanoma, older adults may experience worse progression-free survival due to reduced levels of tumor-infiltrating lymphocytes. Understanding immune-regulatory functions is critical to implementing effective targeted immunomodulatory strategies for elderly patients.

Furthermore, age-related genetic differences such as single nucleotide variants (SNVs) and copy number variants (CNVs) have also been observed in older cancer patients. In patients with squamous cell carcinoma of the lung, Meucci et al. (2018) showed that the defective DNA mismatch repair (MMR)-related signature 6 (SI6) was negatively correlated with patient age. Another step towards targeted, personalized medicine for older cancer patients would be to use gene expression profiling to improve prognostication and thus more appropriate treatment selection.

How to fight diagnostic and therapeutic nihilism in elderly cancer patients

Geriatric screening and assessment

One of the most commonly applied assessment tools for frailty is the comprehensive geriatric assessment (CGA) which evaluates functional status, comorbid medical conditions, cognition, nutritional status, psychological state, social support and the patient's medication. CGA is a systemic evaluation tool, which, if correctly implemented, can help identify and optimize age-related vulnerabilities, reduce over- and under-treatment, and guide treatment recommendations in older patients International consensus guidelines on the management of older patients with various cancers recommend the use of CGAs, e.g., the International Society of Geriatric Oncology (SIOG), American Society of Clinical Oncology (ASCO), and the National Comprehensive Cancer Network (NCCN) guidelines (Table 1). Table 2 shows examples of scales that can be assessed as part of the CGA. The 9-point validated Clinical Frailty Scale (CFS) evolved from the Canadian Study of Health and Aging and is one of the broadest and most commonly used scales in Switzerland and worldwide. It summarizes the level of fitness or frailty of an older adult after evaluation by a healthcare professional from 1 ("very fit") to 9 ("terminally ill"). However, CGAs such as the CFS are not consistently implemented, and more prospective studies are needed to confirm their impact in guiding treatment decisions in elderly patients with cancer.

Domains	Scales
Functional status	Functional Independence Measure (FIM); Barthel Index (BI); ECOG-Performance Status; Katz basic activities of daily living (ADL) scale; simplified Lawton's instrumental activities of daily leaving (IADL) scale
Comorbidities	Charlson comorbidity index
Medications	Number, type, indication
Cognitive function	Folstein Mini-Mental State Examination (MMSE), Schultz-Larsen MMSE; MMSE and/or clock test
Geriatric syndrome	Repeated falls, fecal and/or urinary incontinence
Depression/mood	Geriatric Depression Scale 5, Emotional questionnaire
Nutrition	Body mass index; Nutrition Risk Screening (NRS)
Mobility	Timed Up and Go test
Situational assessment	Accessibility of services, mobility, social environment, accessibility of homerooms

Table 1. Examples of domains and scales used in a Comprehensive Geriatric Assessment (CGA). Adapted from Le Caer et al. 2016.51

Disease	Specialist society recommendations	Reference
CLL	SIOG	52
MM	SIOG	53
DLBCL	SIOG	54
Prostate cancer	SIOG	55,56
Colorectal cancer	SIOG	57
Lung cancer	EORTC	58
Breast cancer	SIOG, EUSOMA	59,60

Table 2. List of guidelines/recommendations for specific cancer types.

The major barrier to completing the CGA is the length of time required to complete the entire assessment (approx. two hours). In other settings, a lack of awareness or expertise may limit its application. In settings where CGA is not feasible or resources are limited, shorter geriatric assessment (GA) tools have been developed. Screening using the relatively quick and easy-to-apply GA tests can help identify vulnerable patients most likely to benefit from a full CGA assessment before starting treatment; therefore, GA screening tests can help preserve clinical time and personnel resources. Validated GA screening tools in the evaluation of physical fragility include the Geriatric 8 (G8) questionnaire, abbreviated Comprehensive Geriatric Assessment (aCGA), Triage Risk Screening Tool (TRST), and Vulnerable Elders Survey-13 (VES-13), many of which can be completed in less than six minutes. GA screening can also be targeted, e.g., to older cancer patients. Nowadays, cancer-specific GA screening tools have been developed, including the Cancer Aging Research Group (CARG) and the Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH), which estimate toxicity risk in older patients receiving chemotherapy. Healthcare professionals can use cancer-specific GAs to develop treatment plans and tailor management strategies to improve QoL for older patients with cancer. To meet the needs of underserved older adults with cancer in rural communities or to minimize infection risk in highly susceptible populations (e.g., from Covid-19), adaptation and delivery of a GA via telehealth is being evaluated as a potential GA tool. GAs are currently available for many different outcome measures such as frailty, nutritional status, comorbidity and cognition.

Cancer patients with pre-existing cognitive impairment may be at great risk for cognitive decline with therapy and are potentially at increased risk for treatment-related adverse events. Cognitive assessment has not historically been routinely incorporated into the evaluation and management of older adults. Several short cognitive screening tools have now been evaluated in cancer patients specifically, including the Montreal Cognitive Assessment (MoCA), the Mini-Mental Status Exam (MMSE), the Mini-Cog80 tests. For example, the Mini-Cog is a short (<3 minutes) cognitive screening tool for dementia that combines a delayed recall item and a clock-drawing test

Cancer-specific GA can be used to guide interventions. Targeted interventions may improve treatment tolerance, adherence, physical function and QoL. The randomized GAIN trial evaluated whether geriatric assessment-driven intervention could reduce chemotherapy-related toxic effects among older cancer patients compared with standard-of-care. The trial included 613 cancer patients with a solid tumor who had started chemotherapy between 2015 and 2019. The median age of the study participants was 71 years (range 65−91). Results from GAIN revealed that implementation of multidisciplinary GA-driven interventions reduces grade 3−5 chemotoxicities in older adults with cancer. Moreover, the trial also showed that GA-driven interventions improved the completion of advance directives. Future research should focus on the validation of GA screening tools in older patients. GA-driven interventions aimed at optimizing treatment should also be applied for this patient population.

Tackling underdiagnosis and undertreatment

Since aging is the single most important risk factor for the development of cancer, it is important to overcome ageism in this field. Professional subjective assessments might lead to older people receiving a reduced range of treatment options, e.g., elderly cancer patients are considered less likely to recover from aggressive therapies and interventions than younger patients.85 Lower risk and less aggressive treatments may be chosen based on the misconception that it will avoid patient morbidity and mortality; however, studies show that such undertreatment leads to a reduced five-year survival for older cancer patients. A recent study showed that 34% of geriatric patients are overtreated while 15% are undertreated. Overtreatment may involve administering standard-of-care to patients who may not gain any clinical benefit. In older patients, overtreatment can lead to more grade 3 and 4 toxicity than treatment appropriately adapted to fragility. In contrast, many studies show that elderly patients often do not receive standard therapy compared to their younger counterparts. Older patients are often considered “frail” and have multiple comorbidities and other characteristics that cause hesitance to prescribe standard regimens. Indeed, although recent studies show that most older patients can benefit from and tolerate intensive cancer therapies, similar to younger patients, there is still a reluctancy to aggressively treat elderly patients, even those without comorbidities. Undertreatment may partly be attributed to common misperceptions about life expectancy, therapeutic benefit and treatment risks. In a study of 397 geriatric patients, 43% received less intensive chemotherapy treatment than the standard-of-care. Assumptions about a patient’s ability to tolerate treatment, QoL, or personal preference should therefore not be based on chronological age. In recent years, the SIOG has begun to address the age-based disparities in research and treatment by advocating assessments of older patients with cancer. Other stakeholders, including the elderly and their caregivers, nurses, palliative healthcare professionals also have an important role to play if the treatment landscape for older cancer patients is to change.

Conclusions and future directions

With an aging population and improvement of life expectancy, managing older people with cancer is a growing burden in Switzerland and worldwide. As newer agents become available and new combinations tested, dedicated studies to identify age-related factors, i.e., immune profiling or predictive/prognostic biomarkers for elderly patients, are warranted. Developing local protocols and guidelines of care for older people with frailty is crucial. Multidisciplinary optimization of personalized treatment plans is required, involving oncology/geriatrics/palliative care/psycho-oncologist/internal medicine/supportive care specialists. Furthermore, establishing a patient registry in the field of geriatric oncology would enable a Switzerland-wide survey of cancer, screening and comparative, comprehensive omics testing, as well as complete tumor profiling, ultimately leading to better survival outcomes and QoL for older cancer patients.

Chapter 12. Cardiological Basel and Baselland multicentric dataset – impact of biological age on decision-making.

"Cardiooncology - biological age guided diagnostic and therapeutic cancer management." Paper in preparation for Aging and Disease, 2023.

Survival analysis

January 2018 – February 2022

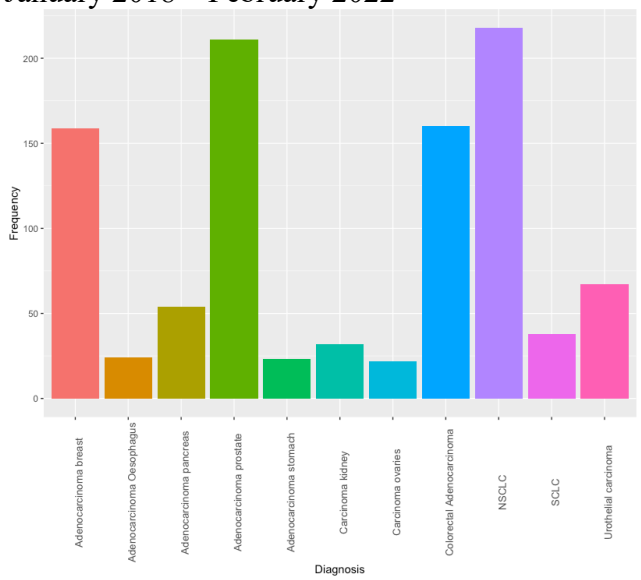


Figure 1. Distribution and frequency of the main diagnoses in the cohort.

Diagnosis Frequency >20 total	Frequency
Adenocarcinoma breast	159
Adenocarcinoma Oesophagus	24
Adenocarcinoma pancreas	54
Adenocarcinoma prostate	211
Adenocarcinoma stomach	23
Carcinoma kidney	32
Carcinoma ovaries	22
Colorectal Adenocarcinoma	160
NSCLC	218
SCLC	38
Urothelial carcinoma	67

Table 1. Distribution and frequency of the main diagnoses in the cohort.

The survival probability of metastatic disease is significantly lower than that of early stage.

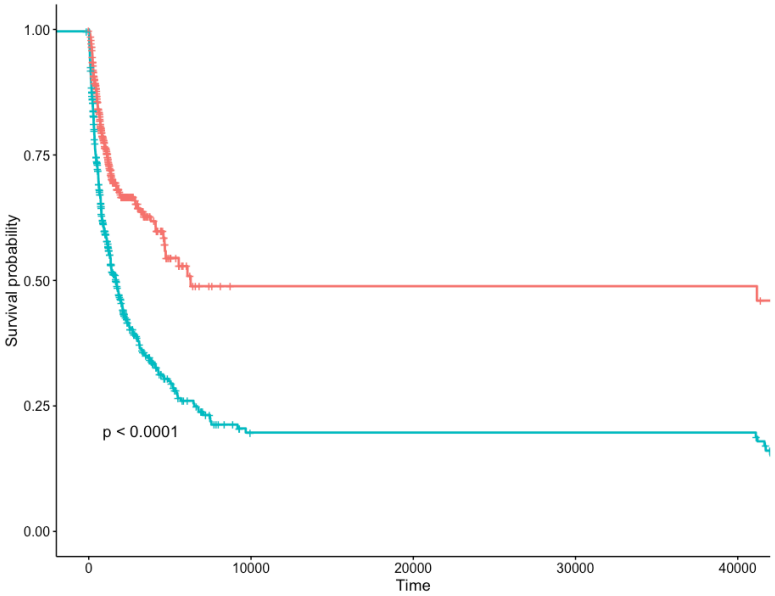


Figure 2. Overall survival analysis for early stage (red) and metastatic disease (green)

The survival probability of female is higher than male

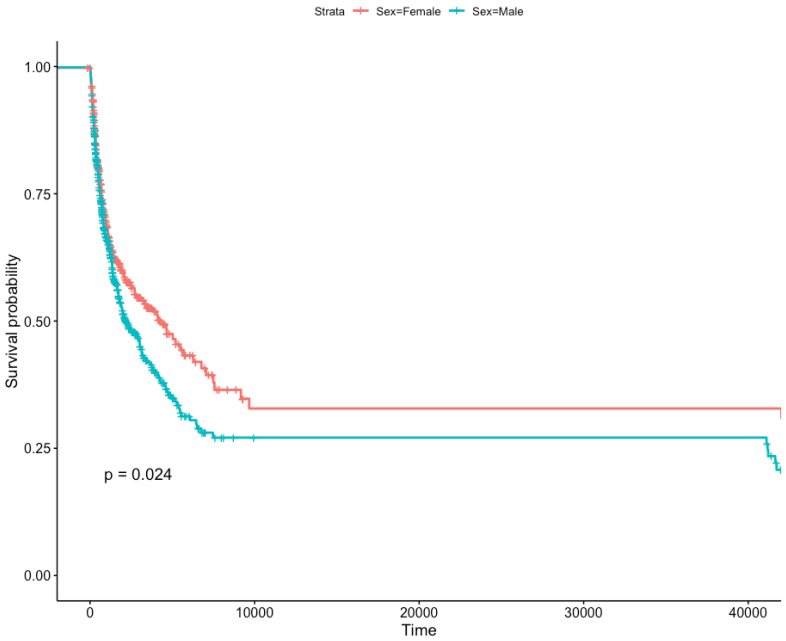


Figure 3. Overall survival analysis for females (red) and males (green).

The survival probability varies considerably between different diseases

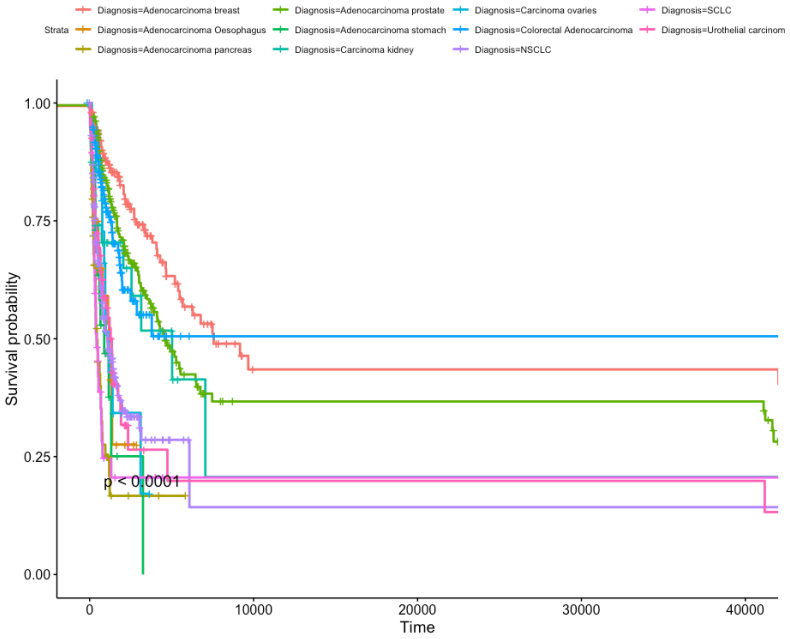


Figure 4. Overall survival analysis based on various diagnoses.

The survival probability can be improved by active treatment.

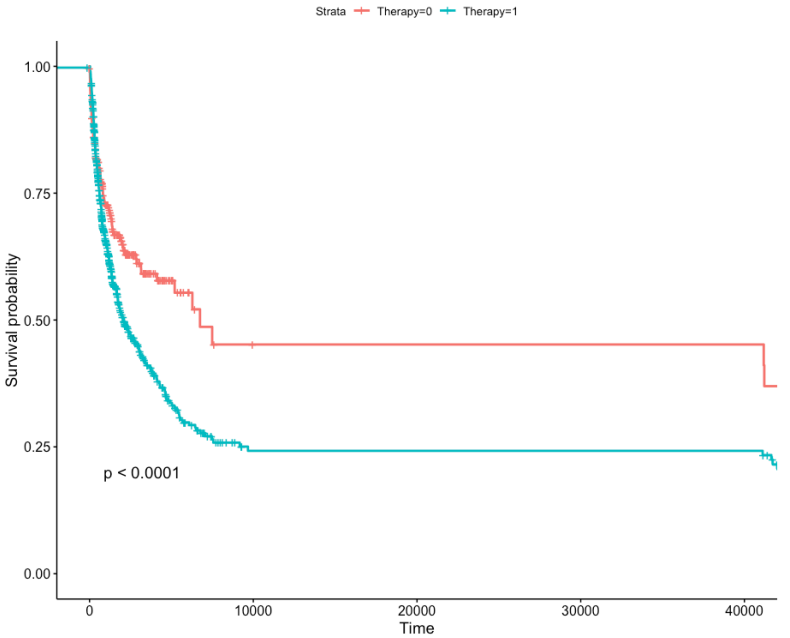


Figure 5. Overall survival analysis for patients without active treatment (red) and with treatment (blue).

Smoking reduces survival probability.

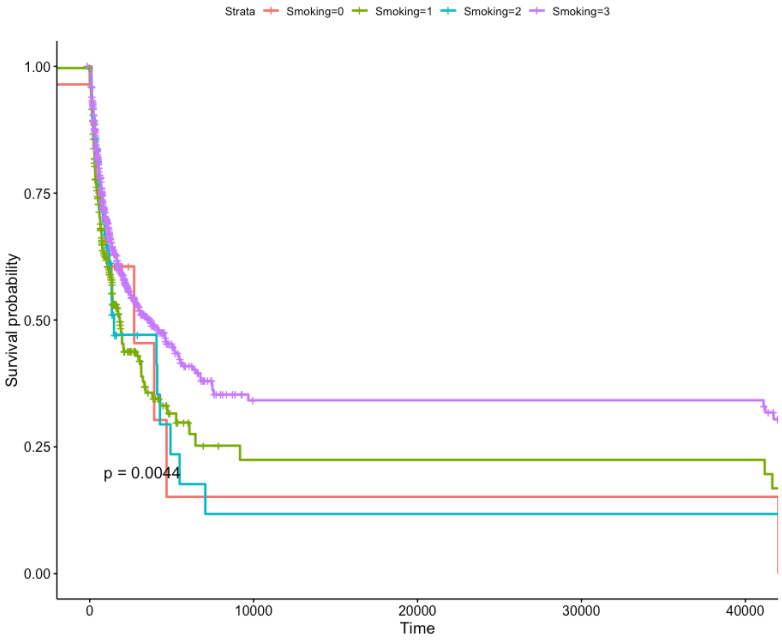


Figure 6. Overall survival analysis based on smoking status.

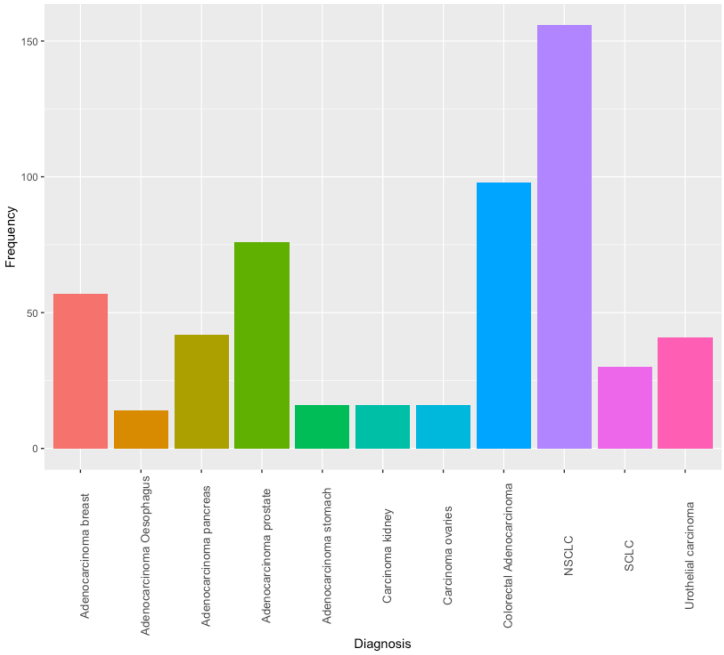


Figure 7. Distribution and frequency of the main diagnoses in the cohort after the year 2018.

Diagnosis (from 1.1.18 on)	Frequency
Adenocarcinoma breast	57
Adenocarcinoma Oesophagus	14
Adenocarcinoma pancreas	42
Adenocarcinoma prostate	76
Adenocarcinoma stomach	16
Carcinoma kidney	16
Carcinoma ovaries	16
Colorectal Adenocarcinoma	98
NSCLC	156
SCLC	30
Urothelial carcinoma	41

Table 2. Distribution and frequency of the main diagnoses in the cohort after the year 2018. NSCLC, adenocarcinoma prostate and colorectal adenocarcinoma are more prevalent in male Adenocarcinoma breast, NSCLC and colorectal adenocarcinoma are more prevalent in female.

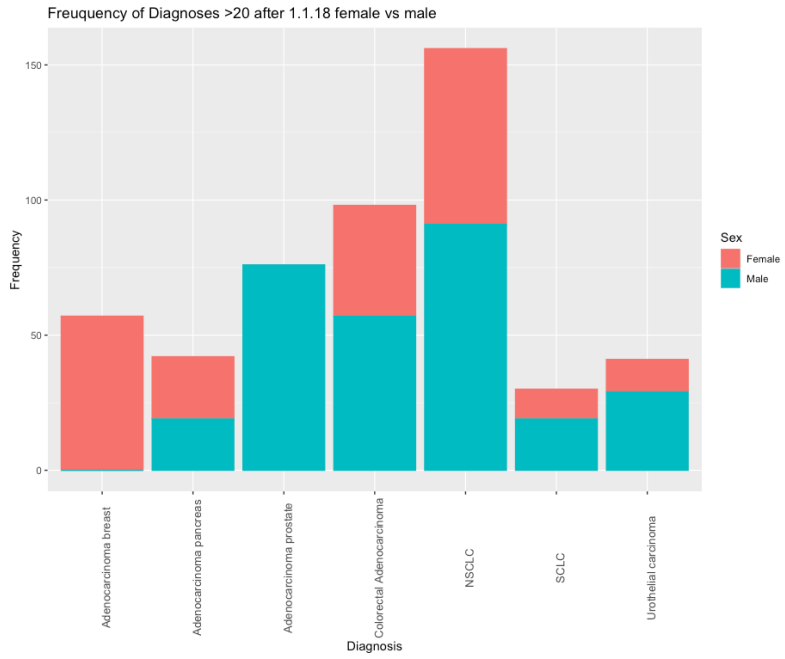
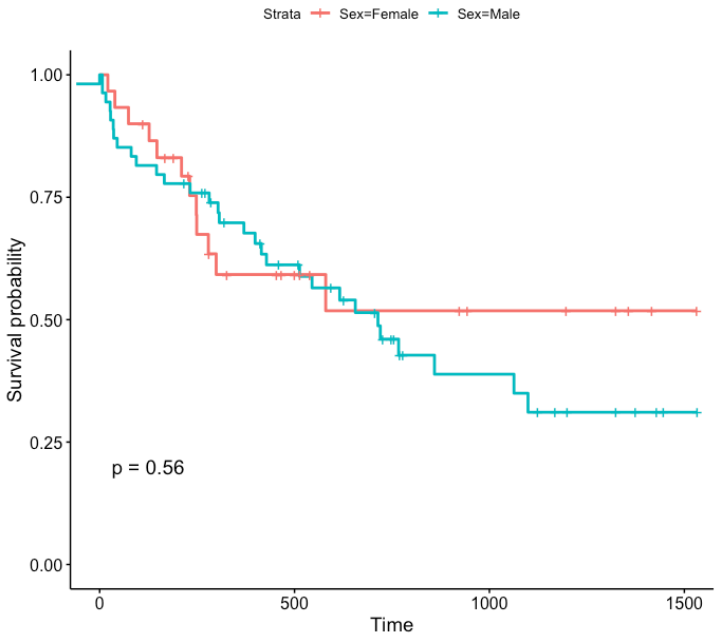


Figure 8. Diagnosis differences for females (red) and males (green) after 2018.

The survival probability in female is higher than male

A



B

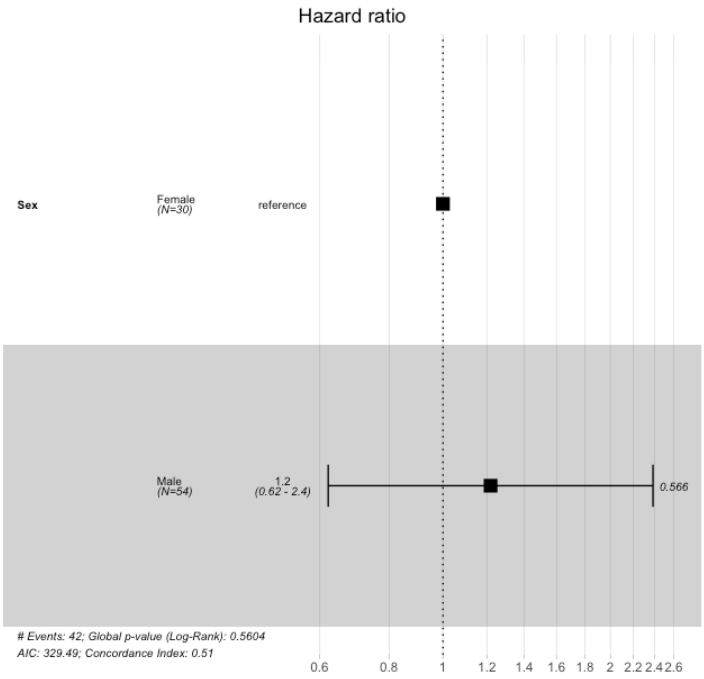
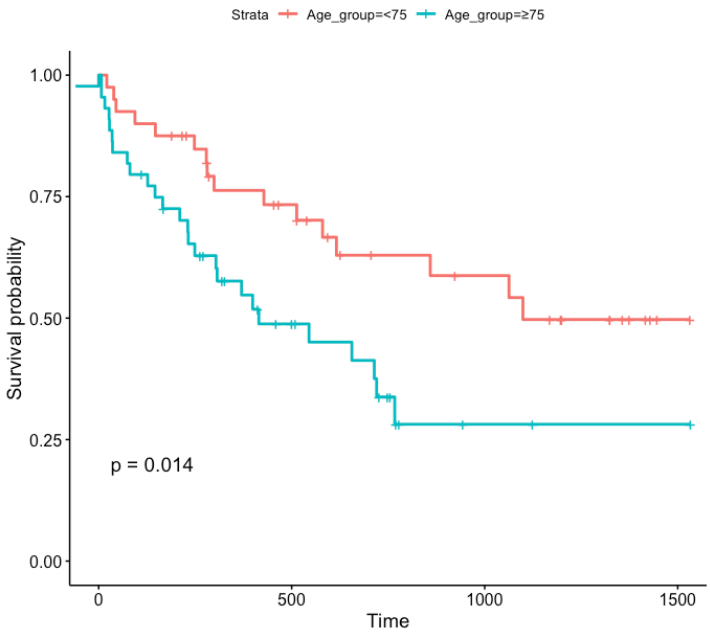


Figure 9. Overall survival analysis (A) and associated Hazard ration (B) or females (red) and males (green) after 2018.

The survival probability in group(age≥75) is higher than group(age＜75)

A



B

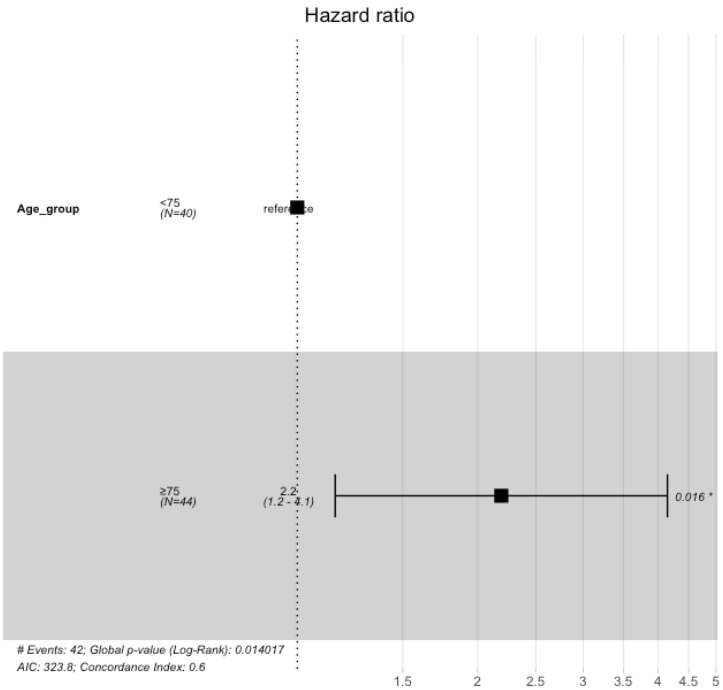
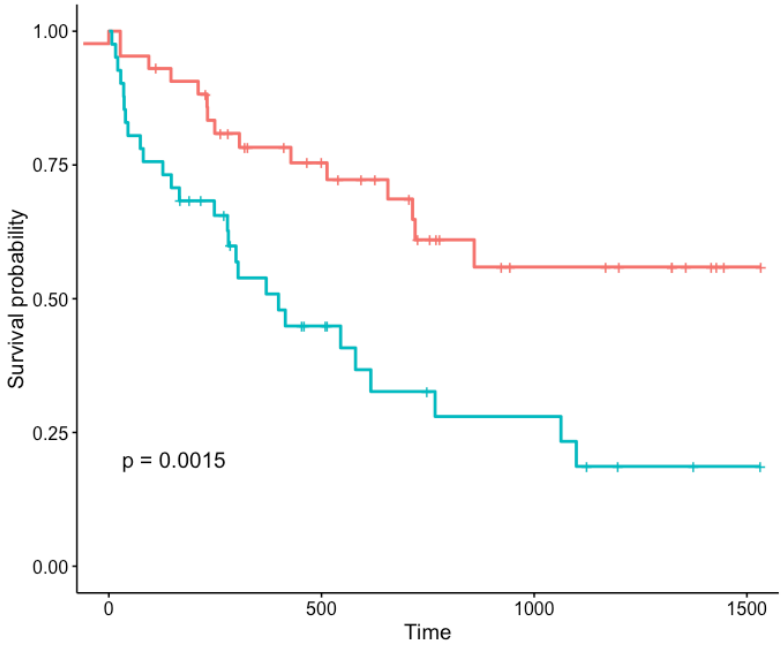


Figure 10. Overall survival analysis (A) and associated Hazard ration (B) for various age groups after 2018.

The survival probability of metastatic disease is lower than early disease.

A



B

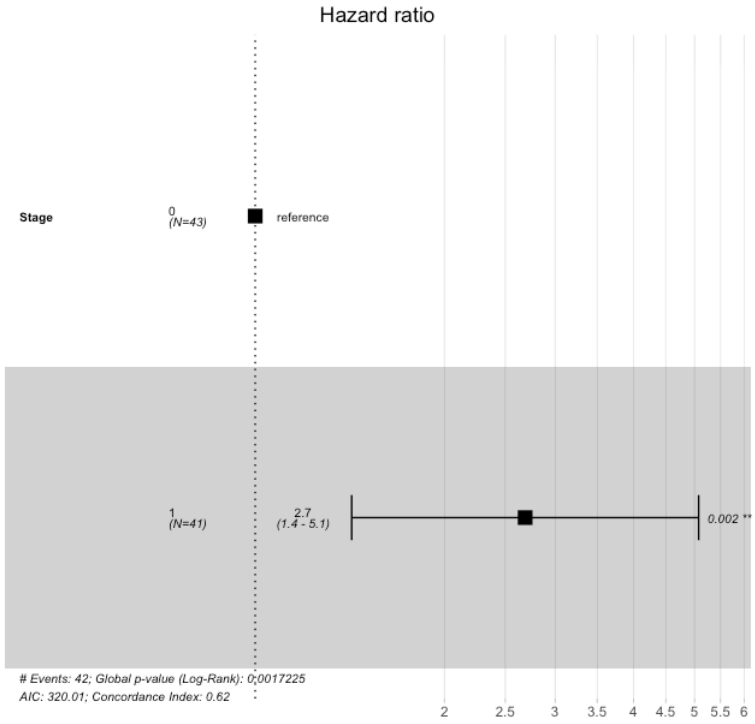
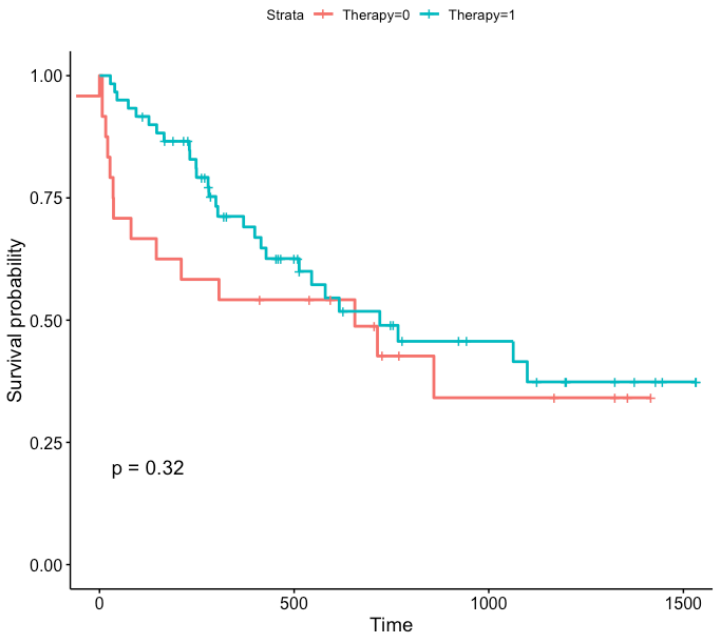


Figure 11. Overall survival analysis (A) and associated Hazard ration (B) for early (red) and metastatic disease (green) after 2018.

Patients receive active therapy have significantly higher survival probability in short term.

A



B

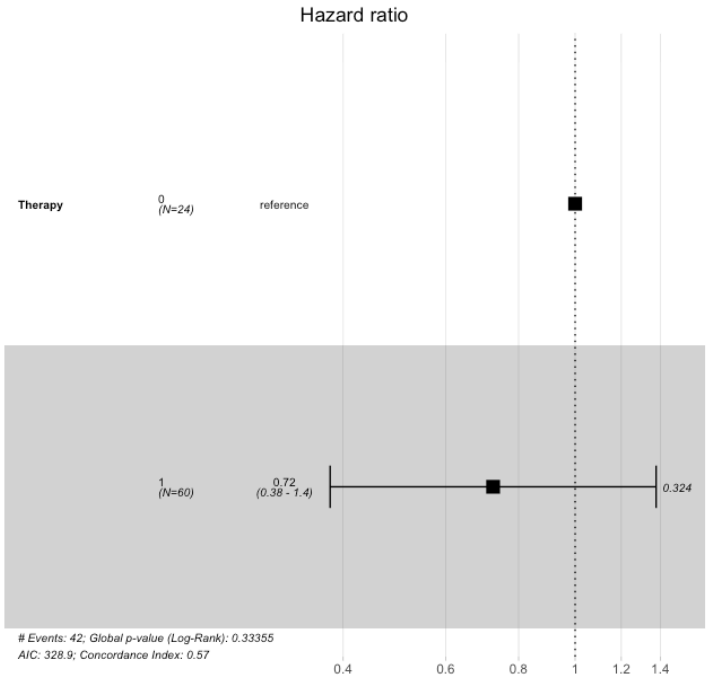
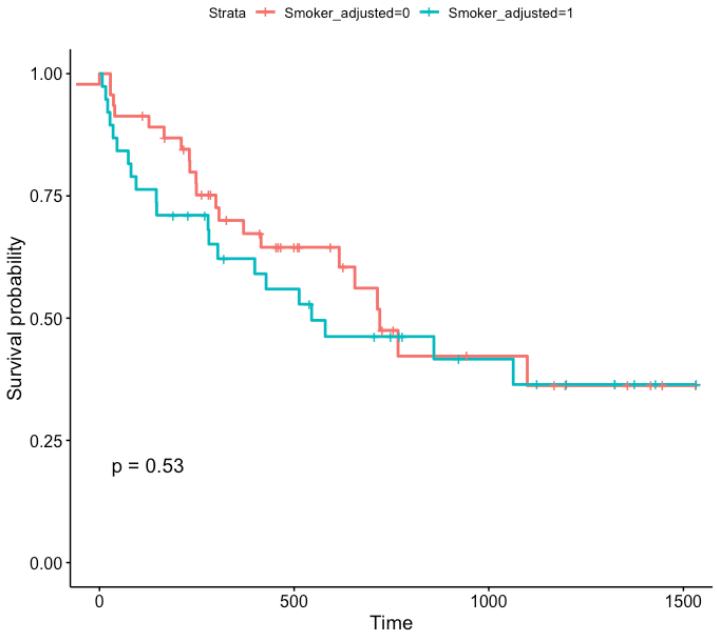


Figure 12. Overall survival analysis (A) and associated Hazard ration (B) for no therapy (red) and active applied therapy (green) after 2018.

The survival probability of smokers is higher than non-smokers

A



B

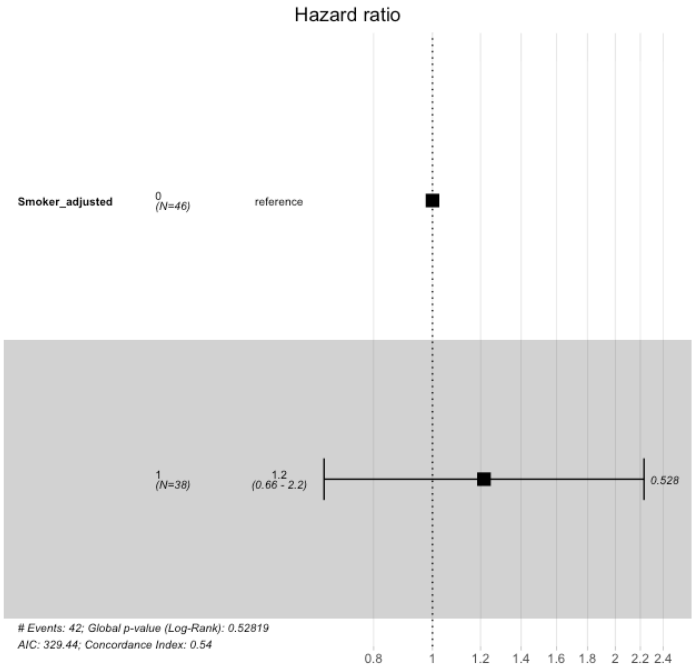
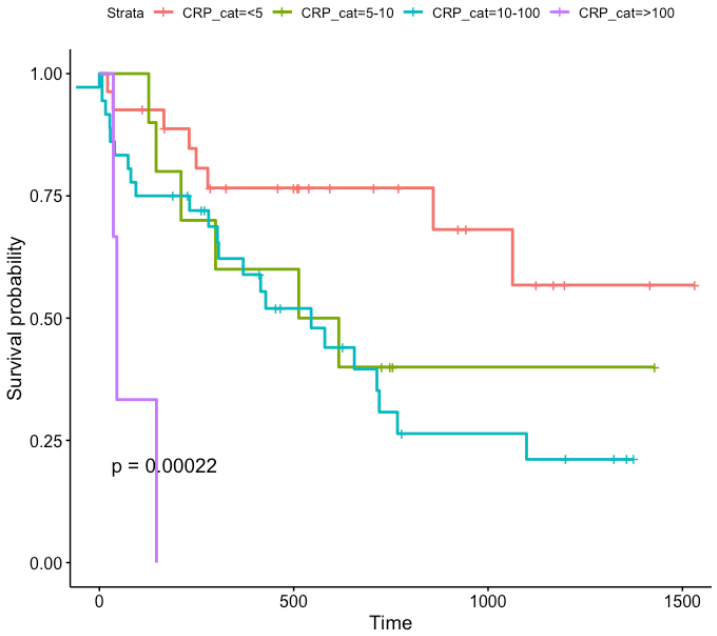


Figure 13. Overall survival analysis (A) and associated Hazard ration (B) for non- smokers (red) and smokers (green) after 2018.

CRP value is correlated with survival probability, with higher the C-reactive protein, the lower the survival probability.

A



B

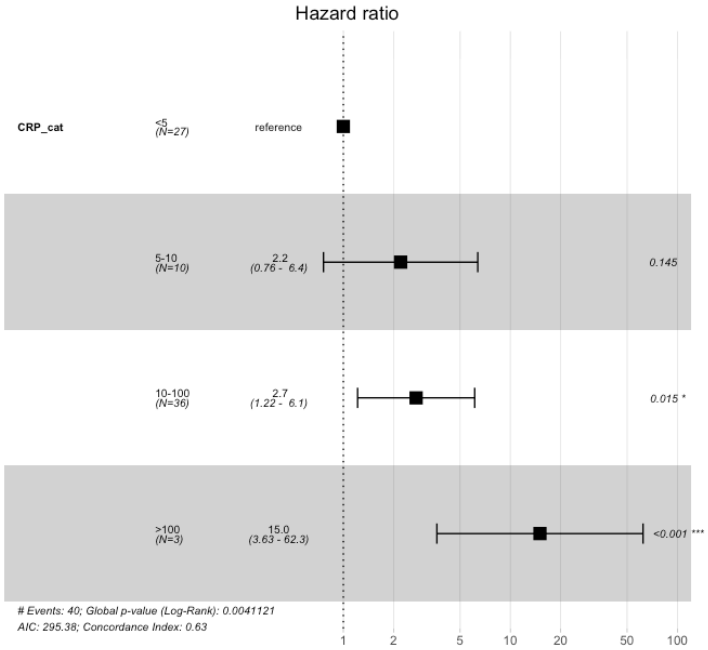
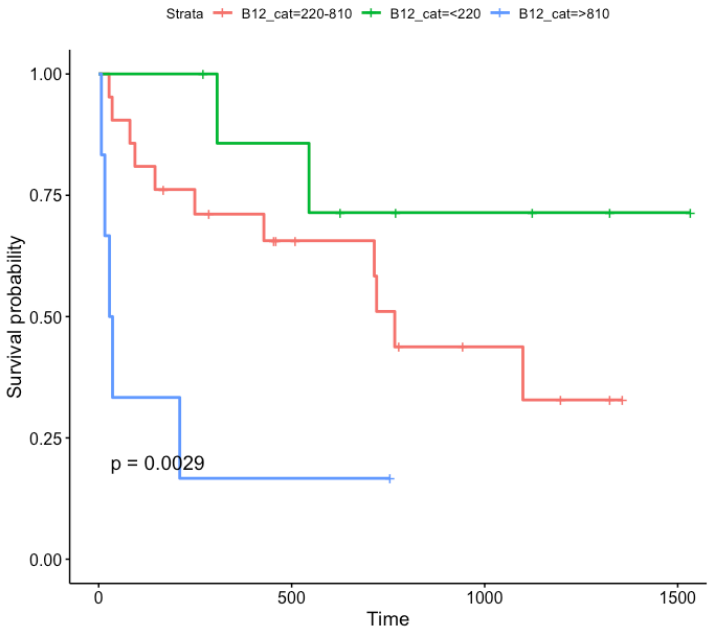


Figure 14. Overall survival analysis (A) and associated Hazard ration (B) for various CRP status.

Vit B12 level is correlated with survival probability, with higher the Vit B12 level, the lower the survival probability.

A



B

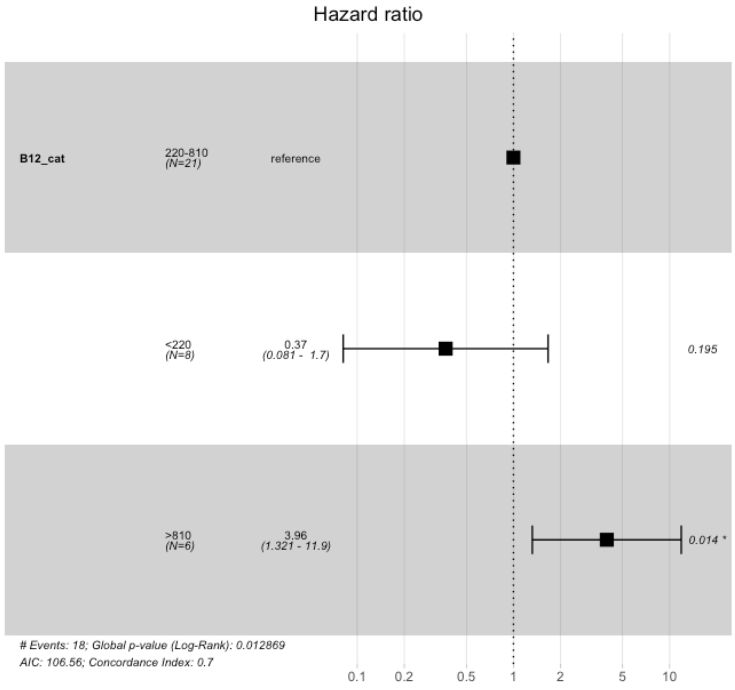
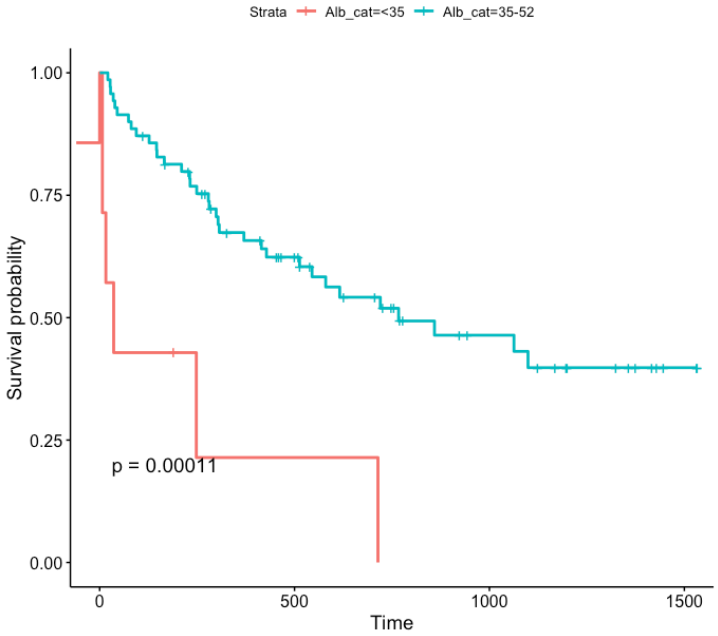


Figure 15. Overall survival analysis (A) and associated Hazard ration (B) for various Vit B12 level status.

Albumin level is correlated with survival probability, with lower the albumin level, the lower the survival probability.

A



B

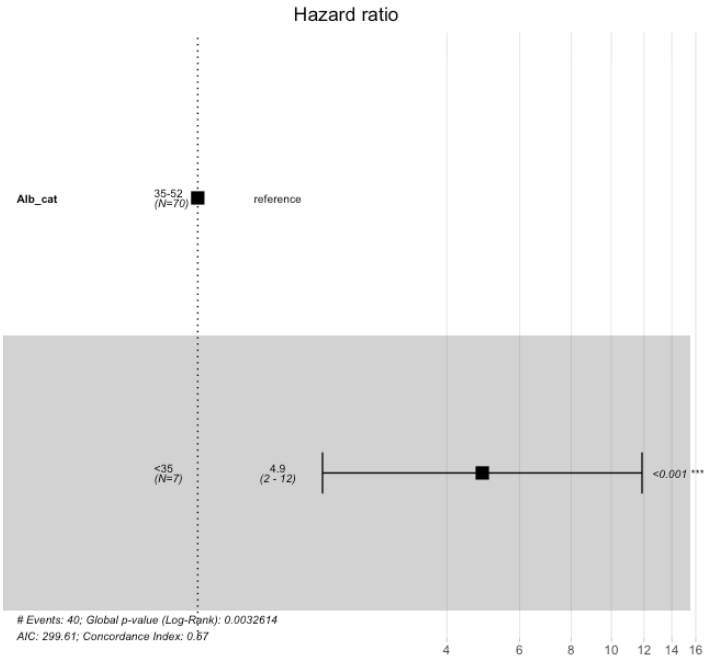
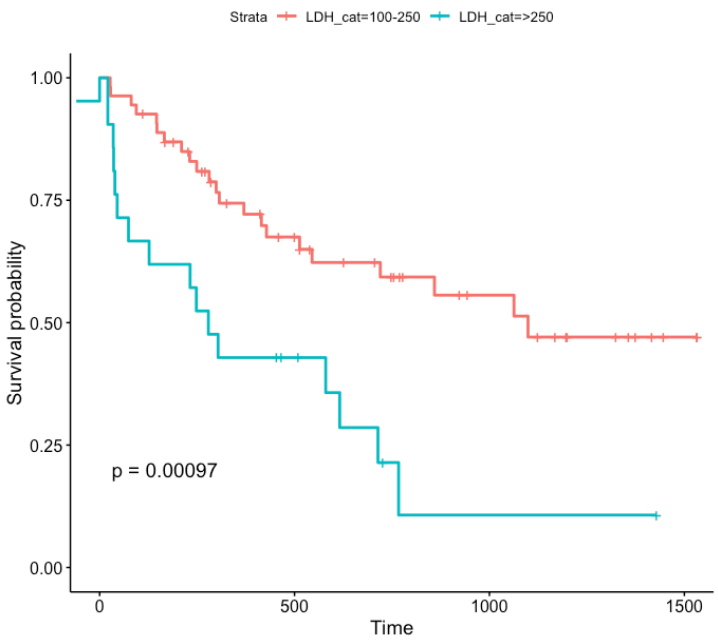


Figure 16. Overall survival analysis (A) and associated Hazard ration (B) for various Albumin level status.

LDH level is correlated with survival probability, with higher the LDH level, the lower the survival probability.

A



B

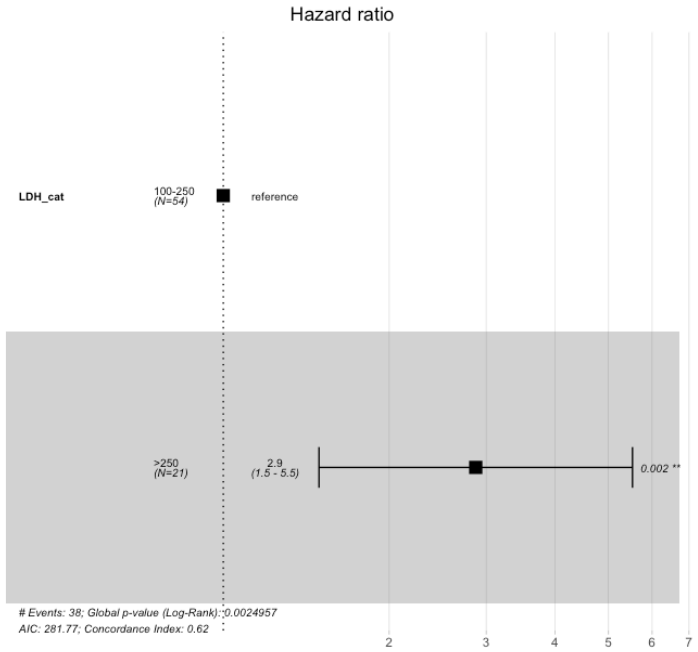
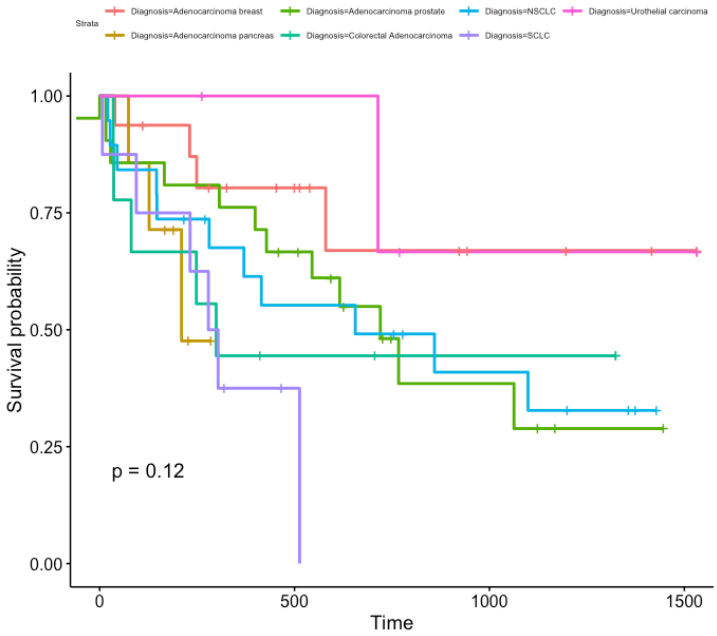


Figure 17. Overall survival analysis (A) and associated Hazard ration (B) for various LDH level status.

SCLC, adenocarcinoma prostate and NSCLC have low survival probability.

A



B

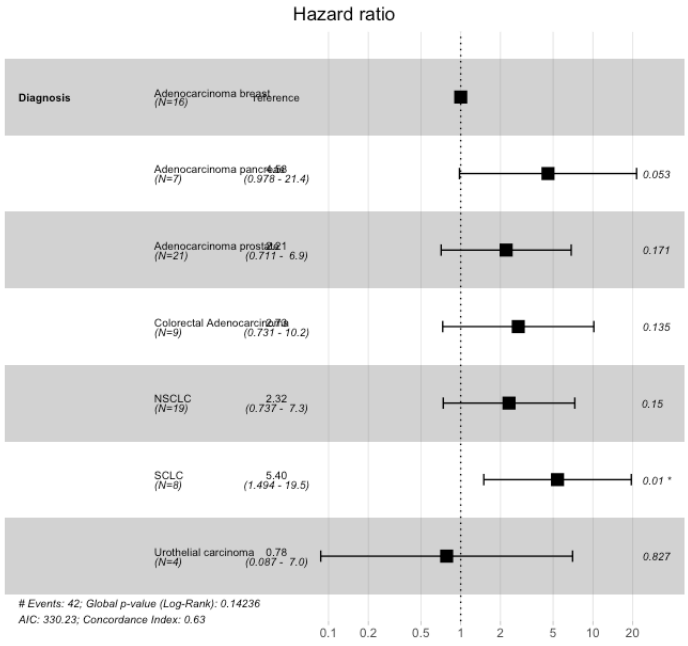


Figure 18. Overall survival analysis (A) and associated Hazard ration (B) for various diagnoses.

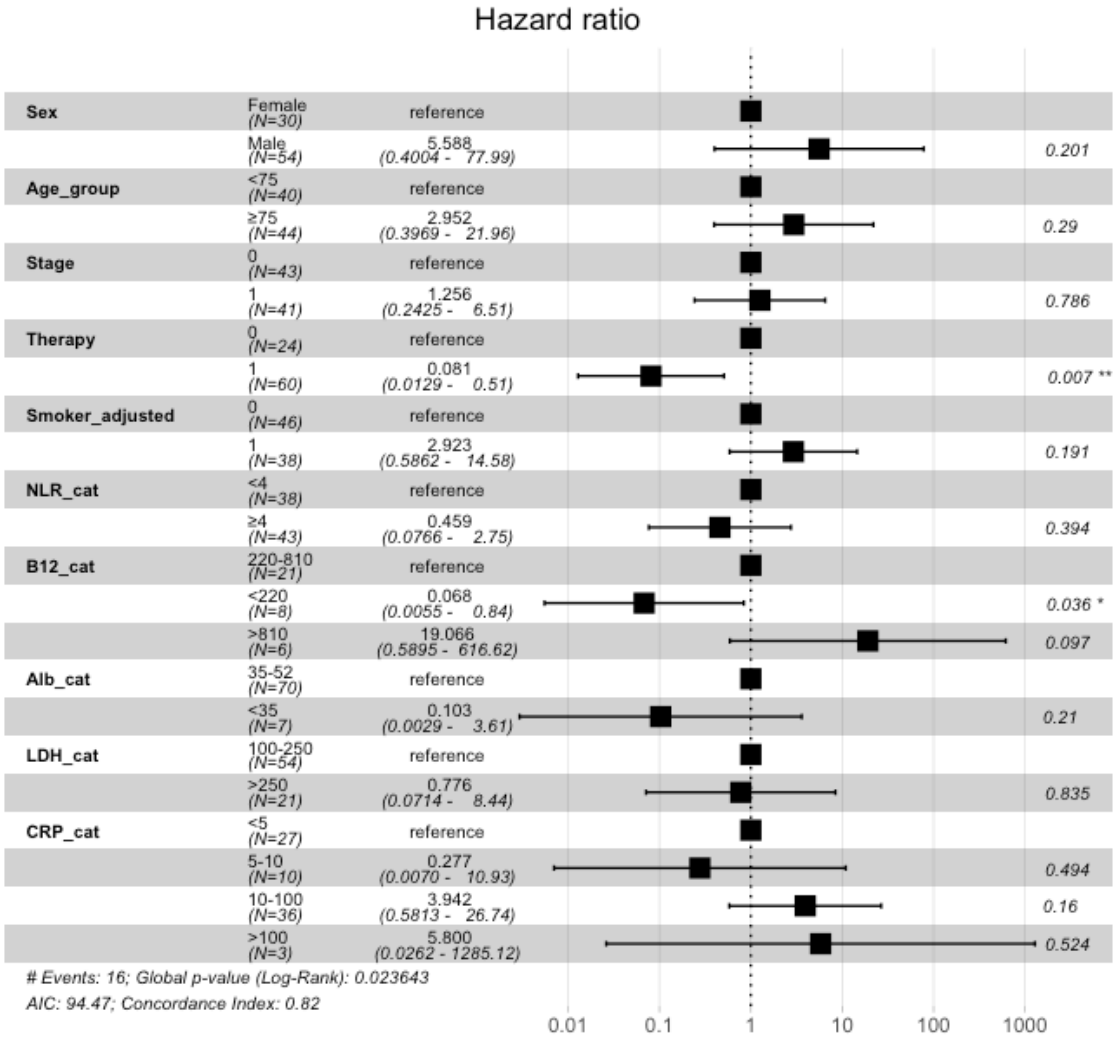


Figure 19. Hazard ration for various BloodAge algorithm markers.

Part V. Gender and biological sex as objective clinical variables

Chapter 13. Gender and biological sex as medical variables in internal medicine and cardiology.

Gender and biological sex are increasingly recognized as significant medical variables with implications for clinical practice in the fields of internal medicine and cardiology. Research has revealed notable differences in the prevalence, presentation, pathophysiology, and treatment response between males and females in various cardiovascular conditions. Understanding the impact of gender and biological sex on disease manifestation and outcomes is essential for optimizing patient care and improving health outcomes. The investigation of gender-specific risk factors, sex-related hormonal influences, and the interplay of genetic and environmental factors contributes to a more comprehensive understanding of disease mechanisms. Furthermore, gender-specific diagnostic and therapeutic strategies can enhance the effectiveness and safety of medical interventions. However, despite advancements, there is a need for greater awareness and integration of gender and biological sex considerations into clinical practice guidelines, medical education, and research design. Future studies should focus on elucidating the complex interrelationships between gender, biological sex, and disease processes to advance precision medicine and promote equitable healthcare for all individuals.

Chapter 14. Gender and biological sex dimensions – awareness of physicians.

Awareness of Sex and Gender Dimensions among Physicians: European Federation of Internal Medicine Assessment of Gender Differences In Europe (IMAGINE) Survey. Bischof E, et al. Internal and Emergency Medicine 2022 October; 17(5):1395-1404.

ABSTRACT

Background: Sociocultural gender is a complex construct encompassing different aspects of individuals' life, whereas sex refers to biological factors. These terms are often misused, although they impact health differently. Recognizing the role of sex and gender on health status is fundamental in the pursuit of a personalized medicine. Aim of the study was to investigate the awareness of the impact of sex and gender on health among European internists.

Methods: European clinicians from 33 European Federation of Internal Medicine countries, participated between January 1st, 2018 and July 31st, 2019. Internists' awareness and knowledge on sex and gender in clinical medicine was measured by a 7-item survey.

Results: A total of 1,323 European internists responded to the survey. 57% were young or middle-aged (78%) women, practicing in public general medicine services (74.5%). The majority (79%) recognized that sex and gender are non-interchangeable terms, though a wide discrepancy exists on clinicians' knowledge of what sex and gender concepts incorporate. Biological sex and sociocultural gender were recognized as determinants of health mainly in cardiovascular and autoimmune/rheumatic diseases. 80% of respondents acknowledged the low female participation in trials and more than 60% the lack of sex-specific clinical guidelines. The majority expressed the willingness of acquiring knowledge on sex and gender impact in internal medicine.

Conclusions: Biological sex and sociocultural gender are factors influencing health and disease. Knowledge translation remains a cross-cutting issue that should be mainstreamed across all European internists. Providing specific education can foster clinicians' personalized approach in clinic and research.

INTRODUCTION

Recently, physicians have been witnessing a paradigm shift in clinical practice from disease-centered to patient-centered approach in the pursuit of personalized medicine. The concept of sociocultural gender, as opposed to biological sex, emerged and gained prominence in all domains of life, including health and disease. It became apparent that sociocultural gender, encompassing different aspects of individuals life including identity, role, relations and institutionalized gender, has an impact in shaping the health of individuals at least equal or even beyond the biological sex. The promotion of these concepts in clinical medicine is pivotal to build personalized and tailored approaches for prevention, diagnostics, therapeutics and disease management. The intersectionality of sex and gender is an important yet neglected determinant of health which deserves attention. Accordingly, the incorporation of a sex (i.e. biological factors) and gender (i.e. psycho-socio-cultural-factors) lens in clinical research and practice is promoted internationally as a promising strategy to better science and to improve clinical and patient-relevant outcomes. To achieve a medical awareness towards adopting sex- and gender-informed decisions in clinical practice, the first step is to provide extensive evidence on their impact on diseases through clinical studies. A first obstacle clinicians have to face is that women are commonly underrepresented in clinical trials with a lack of sex-disaggregated data, thus the feasibility of a sex-specific clinical decision-making is limited. The assessment of gender domains is even more challenging as there is no a standardized measure of gender. Nevertheless, when gender-related factors are collected, sociocultural gender is able to predict outcomes to a greater extent than biological sex alone.

Given this magnitude of sex and gender effects, it is of utmost importance to assess the level of knowledge about the sex and gender dimensions among physicians to better identify areas where improvements can be pursued through interventions such as tailored education. Therefore, the aim of this study was to systematically investigate the knowledge and awareness of the internal medicine community in Europe on sex and gender dimensions in approaching clinical and research questions.

METHODS

The European Federation of Internal Medicine (EFIM) – the largest scientific society of Internal Medicine in Europe – has created a dedicated working group to approach this issue: the Internal Medicine Assessment of Gender differences in Europe (IMAGINE) working group. The design and the goals of IMAGINE working group have been previously published**.** The first planned of IMAGINE was to verify the awareness of sex and gender-related differences on a random sample of resident or specialized internists in Europe. For this reason, a short anonymous online survey was administered to clinicians affiliated with EFIM.

The IMAGINE survey

The IMAGINE survey was designed as an on-line short questionnaire to understand how the sex and gender dimensions are considered and perceived among internists. The study was exempted by Ethic Committee approval because of the anonymous nature of the survey. Participants provided their online written informed consent before filling in the survey.

The short online multiple-choice survey was composed by seven questions (Supplemental Figure 1). Briefly, the first 3 questions aimed at assessing the knowledge on terminology (i.e.

sex vs. gender) and the awareness of factors specifically related or not to sex and gender dimensions. The fourth question explored the perceived knowledge on sex and gender differences in major diseases within the field of internal medicine. The fifth and sixth questions sough to identify if physicians usually look in clinical guidelines for the presence of recommendations specifically tailored according to sex and whether they are aware of the low rate of women's enrolment in clinical trials. Finally, the seventh question targeted the identification of high-priority topics for the internal medicine community in terms of willingness to acquire knowledge in a sex- and gender-perspective. The questionnaire was transferred on a freely available digital platform and circulated as an electronic link through an e-mail distribution list, as detailed below.

Survey Target Population and Dissemination Strategy

The survey was circulated strategically among all members of EFIM between January 1st 2018 and July 31st 2019. Briefly, EFIM encompasses 35 internal medicine societies among 33 countries. The respective IMAGINE WG member (representative of each country) was also the national coordinator of the study and responsible for the country-specific dissemination of the survey via their national society network, via direct links to the hospitals or via hospital representatives. In addition, professional social media networks of the EFIM were used to popularize the survey. All professionals had to give mandatory information such as: (i) age (years) and sex; (ii) practicing country; (iii) professional position status; and (iv) specialty/subspecialty and years of practice. The invitation was repeated at least 3 times during the recruiting period.

Statistical Analysis

The average of EFIM active members is 2000 (average of annual congress attendee), the minimum random sample size computed for the present survey would be 1200 subjects, considering a margin error of 5% and a confidence level of 95% and a response distribution of 50%. Random sample of surveyed participants was representative for EFIM affiliated Residents + Specialists in IM. The Imagine Survey was launched during the 2018 EFIM congress in Wiesbaden (Germany).

Continuous variables were expressed as mean and standard deviation (SD) and differences between groups were evaluated according to Student t test or ANOVA test, as appropriate. Categorical variables were expressed as counts and percentages. Differences among the different groups were evaluated by chi-square test. A logistic regression analysis was performed to identify those baseline characteristics associated with the 'Yes/I Don't Know' answer to the question 'Do you think that the terms "Sex" and "Gender" are synonymous?'. After univariate analysis, all those baseline characteristics significantly associated with the answer were included into a multivariable model. Additionally, we also performed a subgroup analysis about male and female respondents regarding question one and question two of the survey. A two-sided p value <0.05 was considered to be statistically significant. All analyses were performed using SPSS v. 25.0 (IBM, NY, USA).

RESULTS

Baseline Characteristics of Survey Responders

The baseline characteristics of the participants are illustrated in Table 1. Overall, 1,323 individuals participated in the survey, with a similar distribution of females (56.9%) and males (42.6%), with a mean (SD) age of 42.3 (12.6) years. The predominant group was that of young age (51.2%, aged less than 39 years), from Western and Southern Europe (over 90%) and engaged as internal medicine health care specialists (85.1%), rather than other specialists or clinical scientists (8%). The mean (SD) amount of practicing years was 15.3 (12.5), with a very similar distribution in categories from 0-4 to 20-39 years (each ~20%).

Gender and Sex Knowledge among IMAGINE Responders

The first question approached the general knowledge about the terms sex and gender and whether these are synonymous. Almost 79% of the surveyed individuals responded correctly (no), however as much as 15% responded incorrectly (yes) and almost 6% did not know the answer.

Regarding the association between the baseline characteristics and the answer 'Yes/I Don't Know' to the first question, univariate analysis found that no characteristic was significantly associated with the answer (Table 1). Hence, the multivariable model was not compiled.

Most participant agreed or strongly agreed on statements assessing the general knowledge of sex vs. gender (Figure 1A), especially over 90% agreed that sex relates to biological factors, gender to psychosocial factors, both are interactively influencing health and their stratification is needed in research planning and in randomized controlled trials. On the other hand, less than half of the respondents (40%) consider sex and gender while prescribing medications in daily practice.

The analysis stratified according to the biological sex of responders showed that, as compared with male responders, female responders more likely to strongly agree about: the significance of the term "gender" (58.0% vs. 54.3%, for females and male respectively; $p<0.001$); the interaction between sex and gender in influencing health (55.2% vs. 50.5%, respectively; $p=0.05$); the role of sex and gender as determinants along life phases (45.3% vs. 40.8%; $p=0.05$); considering sex and gender in the research planning (46.9% vs. 42.6%; $p=0.021$); and that there is a lack of evidence in clinical research about differences between sex and gender (42.8% vs. 29.8%; $p<0.001$) (Figure 1B)

Identification of Sex and Gender-related factors

On average, 50-60% of the participants correctly allocated sex as determinant of body size, genetics, sex hormones, reproductive status and body composition. Only 30-40% correctly allocated gender as determinant of diet, personality traits, marital and socio-economic and working status. As much as 50-60% stated that ethnicity, religion, age, comorbidities, disabilities, environment and geographic location are neither sex nor gender related (Table 2).

Beliefs and Knowledge Interest on Sex and Gender as Determinants of Health and Diseases

Cardiovascular (92.9%), vascular (88.4%), lung diseases (70.9%), and inflammatory bowel disease (68.9%) were most frequently identified as being influenced strongly by sex and gender, while infectious (46.3%), renal (44.1%) and neuropsychiatric diseases (28.0%) were strongly misidentified as not being influenced by sex or gender. The areas where most

participants expressed interest in learning more about sex and gender influence for application to their clinical practice were: cardiovascular (57.4%), vascular (16.7%), inflammatory bowel (6.7%) and neuropsychiatric (6.1%) diseases.

Women Participation in Randomized Clinical Trials for Approval of New Drugs

Among the participants, the 41% responded that the current female representation in clinical trials ranges from 10 to 30% while 44% stated a mean proportion of 50%. Only 64% ever heard about specific recommendations for women in guidelines.

DISCUSSION

The main result of the IMAGINE survey is that one fifth of European residents or specialized Internists use the two terms "sex" and "gender" interchangeably. Even though most physicians stated that they are aware of the influence played by sex and gender in healthcare practice and research planning, they were not able to correctly identify what these concepts represent. Consequently, they do not actively apply that knowledge when it comes for example to medical prescriptions. This situation may be a consequence of the underrepresentation of women in randomized controlled trials for approval of new drugs and of the lack of specific sex/gender driven guidelines. Indeed, European internists were aware of the lack of evidence. Strikingly it is acknowledged by a large pool of physicians that cardiovascular diseases are strongly influenced by sex and gender, whereas other pathological entities are not.

While biological sex might be primary seen as determining health differences between males and females, gender is primarily implicated in inequalities resulting from varying patterns of social roles, behavior, attitudes and even lifestyles. Both sex and gender are influenced by diverse socio-cultural factors and social stratification, but gender is related to conditioning and stereotyping of personality traits considered typically "feminine" or "masculine", which in turn significantly affect somatic and psychological well-being. Several studies confirmed that there are substantial biological differences between males and females, which drive various diseases and even brain aging, which further influences development of a broad spectrum of diseases and shapes the attitude towards health status. Meanwhile, most guidelines are based on a pre-selected cohort of mostly male representatives (female participation in large randomized controlled trials is as low as 10-30% depending on the health domain). Meanwhile, there is a growing base of reports about major sex differences in pharmacology. Our survey is the largest to date and first European study on sex and gender awareness, knowledge, interest and practice among internal medicine professionals, both trainees and advanced specialists. A specific lack in training and dissemination of the sex/gender issue is a knowledge gap urgently in need to be filled. Therefore, multidimensional gender-based frameworks across all diseases are essential for healthcare professionals.

Gender as psycho-socio-cultural construct, is still underestimated in its effect on the incidence, etiology, and development of diseases and the effectiveness of therapy in everyday clinical practice, given the differentiating impact of various external factors and reactivity of (epi)genome on health. Clearly, this encompasses more than only cardiovascular diseases and endocrinology, but perhaps even more so respiratory diseases, gastro-hepato-enterology, hematology, neurology and autoimmune diseases. This also refers to sex- and

gender-dependent differences in drug response, medication adherence and metabolism. Lastly, there is currently enough evidence that not only biological sex and age, but also sociocultural gender is an independent risk factor for individual diseases, and can largely determine their course.

Another finding of interest is that the intersectionality between sex, gender and other relevant features of individuals requires to be addressed and discussed with the internists' community. Intersectionality involves the study of the ways that race, gender, disability, sexuality, class, age, and other social categories are mutually shaped and interrelated. Information pertaining to how these other social constructs may interact with sex and gender to influence risk of diseases and their clinical progression are warranted to guide internists in providing patient-individualized pathways of care.

Clinical Implications

Despite increasing evidence that an individual's sex is one the most important modulators of disease risk and response to treatment, consideration of the patient's sex in clinical decision making is often lacking. This is a matter of concern as precision medicine, the new paradigm of 21st century, should begin with attention to sex and gender differences. Surprisingly, there is a reduced awareness of biological, physiological and epidemiological differences between the biology behind medicine in men and women. In our survey, we spotlighted how the clinicians' community is aware of low participation of women in randomized controlled trials for testing new drugs and of the drawbacks on clinical guidelines that do not provide sex-specific recommendations.

Internal medicine is one of the core medical specialties. Internists as they are expected to manage and triage their patients to further, more narrow fields of medicine, need to be informed and trained on the latest of medicine progress. As internists' responsibilities are particularly related to complex clinical scenarios and disease patterns (e.g. multimorbidity, senescence, etc.), they should necessarily be familiar with the gender concept and carefully discern its categorically scientific elements and apply it in patient care.

While cardiovascular, neuropsychiatric and immune-endocrine fields have made tremendous advancements in integrating sex and gender in respective research focus, internal medicine is still relatively far from such progress due to the complexity of the field. The IMAGINE WG thus aimed to explore the respective sex vs. gender knowledge and attitudes of European internal medicine community, both in training and at an advanced career stage, conducting the largest survey in Europe thus far. The IMAGINE survey was the necessary premise to understand and learn which are the areas of improvement and the knowledge gaps to tackle among the European internal medicine clinicians. The results of the study will help promote future prospective studies that elucidate differences in diseases' diagnosis and treatment with regard to sex and gender in internal medicine. Finally, the IMAGINE survey highlighted the need of implementing sex- and gender-informed approach in internal medicine training programs.

	Survey Cohort N= 1323	OR (95% CI)	p
***Sex**, n (%)*			
Male	564 (42.6)	1.48 (0.17-12.81)	0.720
Female	753 (56.9)	1.27 (0.15-10.90)	0.831
Other	6 (0.5)	Ref.	Ref.
***Age**, years mean (SD)*	42.3 (12.6)	0.99 (0.98-1.01)	0.401
***Age Classes**, n (%)*			
20-29	180 (13.6)	0.91 (0.33-2.48)	0.857
30-39	498 (37.6)	1.29 (0.51-3.29)	0.588
40-49	252 (19.0)	1.64 (0.65-4.14)	0.291
50-59	232 (17.5)	1.31 (0.53-3.23)	0.562
60-69	126 (9.5)	1.25 (0.48-3.23)	0.645
≥70	35 (2.6)	Ref.	Ref.
***European Region**, n (%)*			
Northern Europe	34 (2.6)	0.88 (0.24-3.24)	0.848
Western Europe	578 (43.7)	2.29 (0.51-10.28)	0.279
Eastern Europe	27 (2.0)	0.84 (0.23-3.09)	0.789
Southern Europe	670 (50.7)	1.82 (0.42-7.90)	0.425
Non-EU Countries	13 (1.0)	Ref.	Ref.
***Work Setting**, n (%)*			
General/Primary Care	1126 (85.1)	Ref.	Ref.
Specialised Care	77 (5.8)	0.89 (0.50-1.59)	0.690
Research Centre	29 (2.2)	-	-
Other	91 (6.9)	1.47 (0.91-2-37)	0.114
***Type of Practice**, n (%)*			
Public	989 (74.8)	1.28 (0.70-2.35)	0.423
Private	37 (2.8)	0.81 (0.29-2.22)	0.678
Public & Private	204 (15.4)	1.12 (0.66-1.92)	0.671
Other	93 (7.0)	Ref.	Ref.
***Role**, n (%)*			
Junior Physician	424 (32.0)	0.90 (0.47-1.75)	0.762
Attending Physician/GP	595 (45.0)	1.17 (0.65-2.13)	0.597
Senior Physician	226 (17.1)	1.25 (0.68-2.29)	0.478
Other	78 (5.9)	Ref.	
***Specialty**, n (%)*			
Internal Medicine/Geriatric	803 (60.7)	0.86 (0.61-1.21)	0.372
Intensive Care	33 (2.5)	1.04 (0.72-1.50)	0.824
Clinical Sub-Specialties	210 (15.9)	1.16 (0.51-2.61)	0.726
No/Other	277 (20.9)	Ref.	
***Practice Years**, mean (SD) 1316*	15.3 (12.5)	0.99 (0.98-1.00)	0.219
***Practice Years**, n (%) 1316*			
0-4	292 (22.1)	Ref.	Ref.
5-9	293 (22.1)	0.79 (0.53-1.18)	0.256
10-19	290 (21.9)	1.05 (0.71-1.54)	0.809
20-39	365 (27.6)	0.87 (0.60-1.26)	0.458
≥40	76 (5.7)	0.57 (0.28-1.14)	0.111

Legend: GP= General Practitioner; SD= Standard Deviation.

Table 1. Baseline Characteristics of Survey Participants

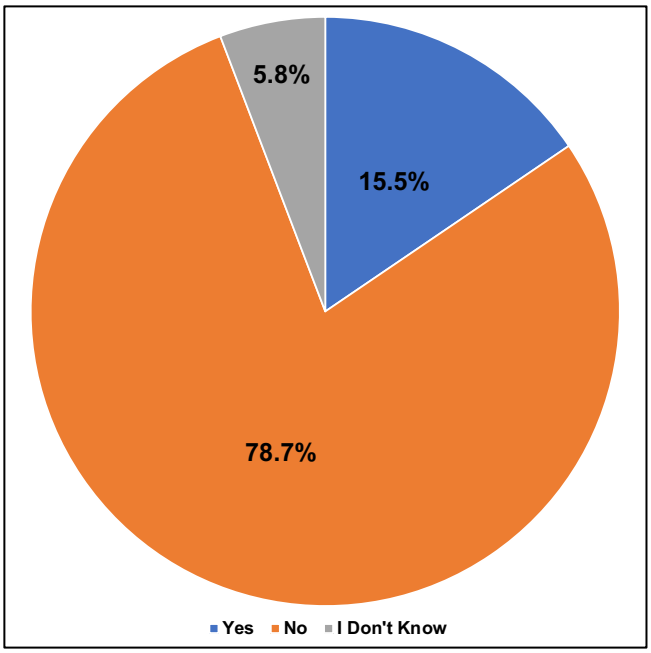


Figure 3. Do you think that the terms "SEX" and "GENDER" are synonymous?

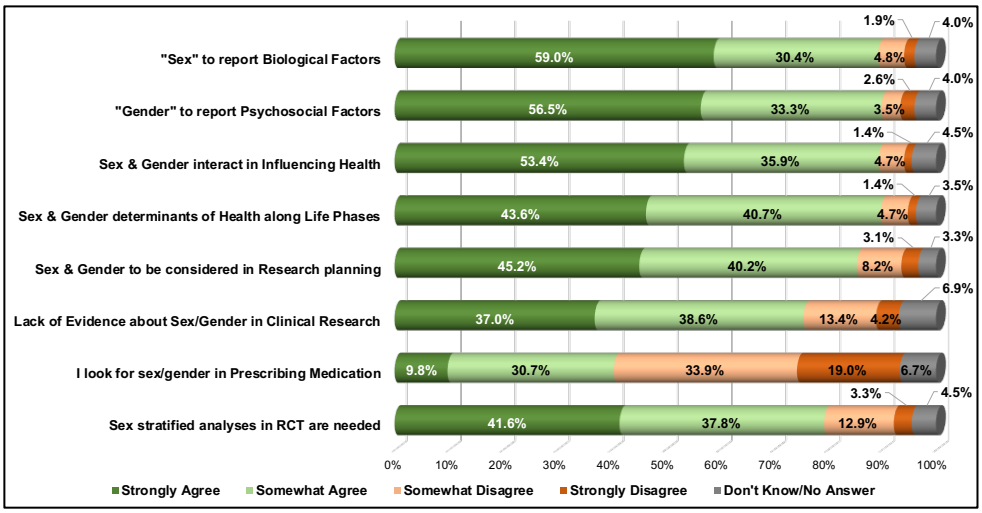


Figure 4. Please read the following statements, and indicate how much you agree or disagree with each one of them

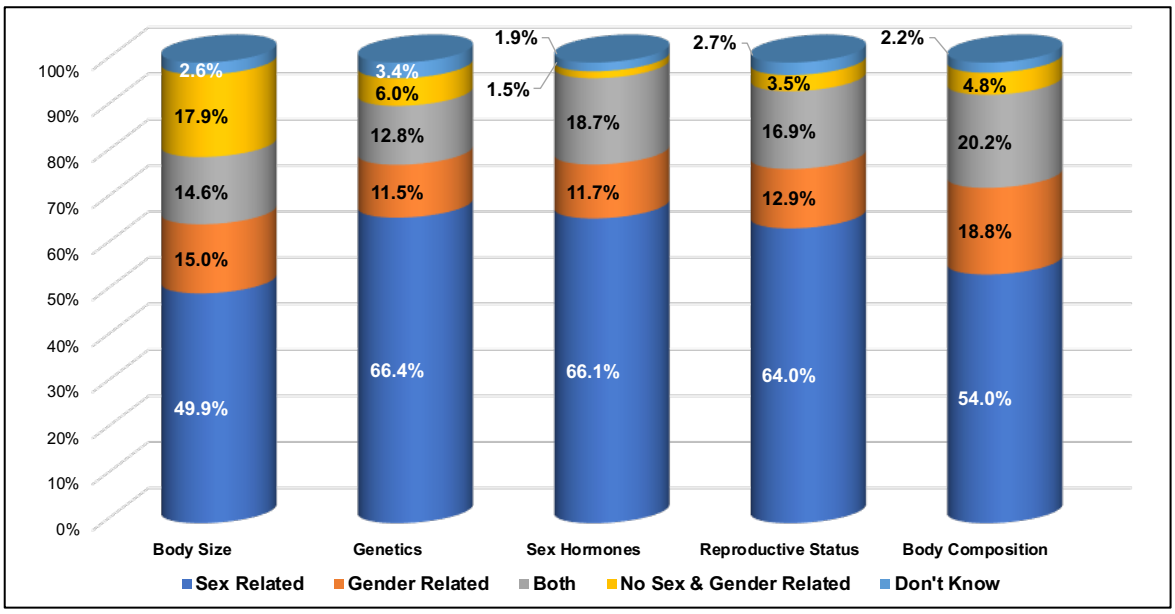


Figure 5. For the following variables select what you think are sex-related, gender-related or both or not?

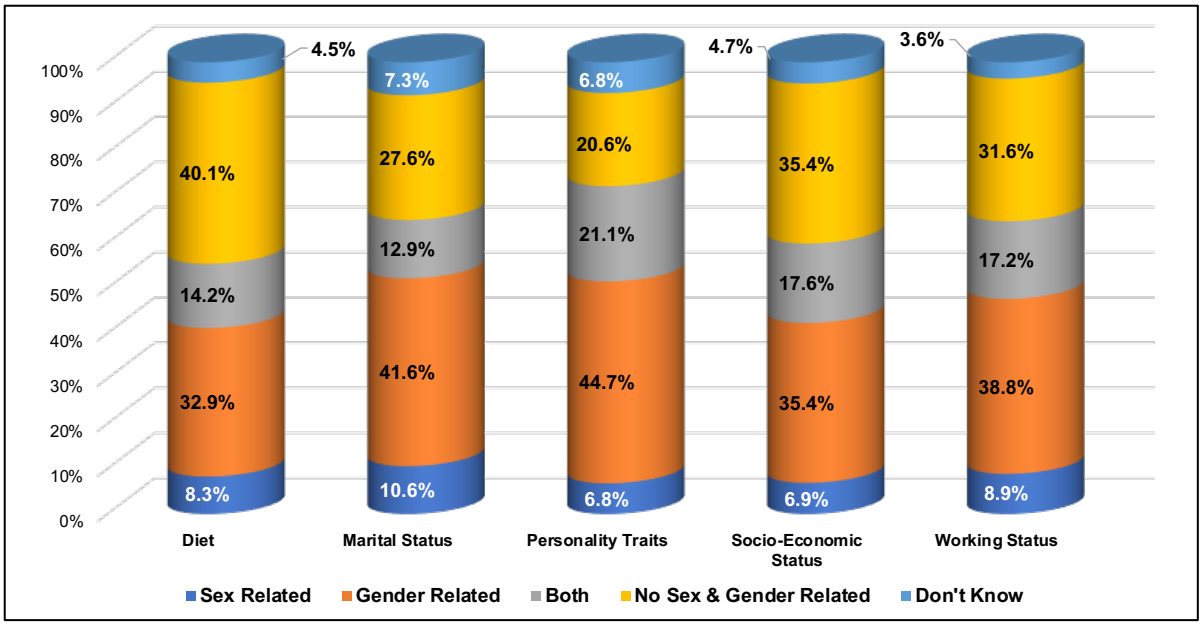


Figure 6. (cont.) For the following variables select what you think are sex-related, gender-related or both or not?

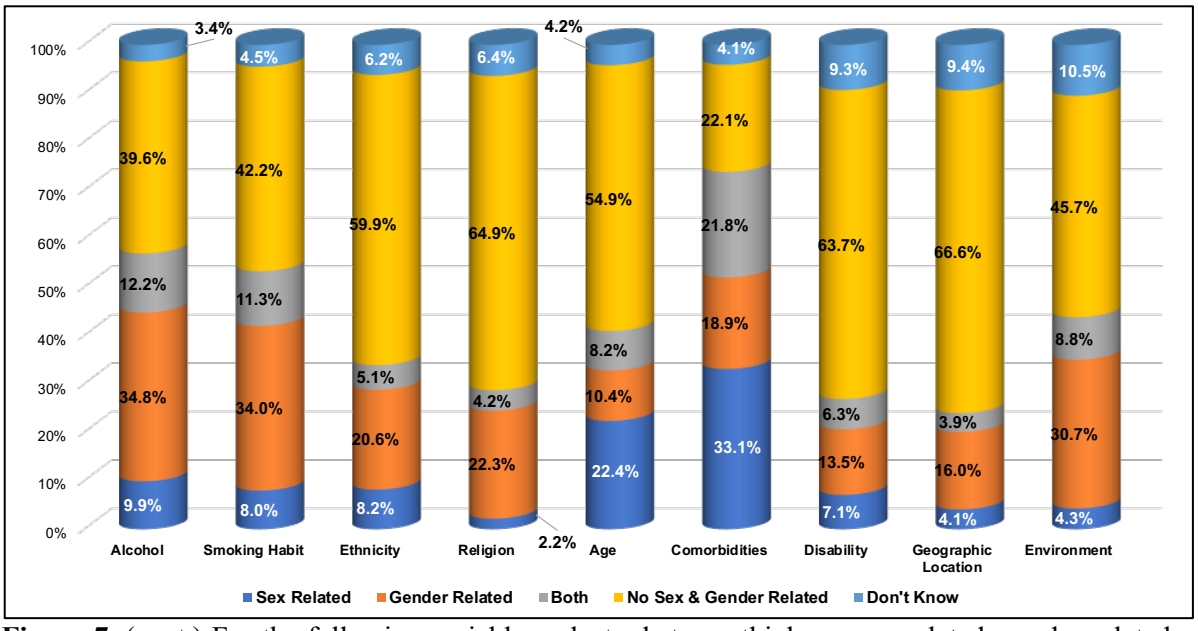


Figure 7. (cont.) For the following variables select what you think are sex-related, gender-related or both or not?

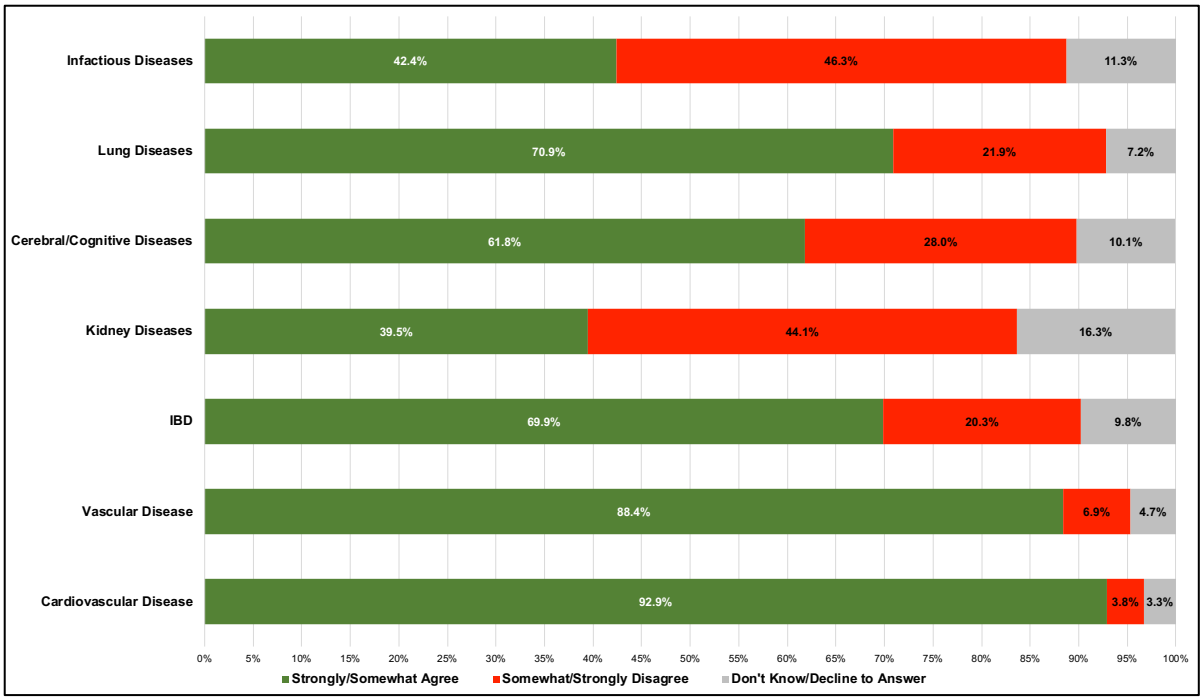


Figure 8. Among these categories of Health and Diseases which one are influenced by sex and gender

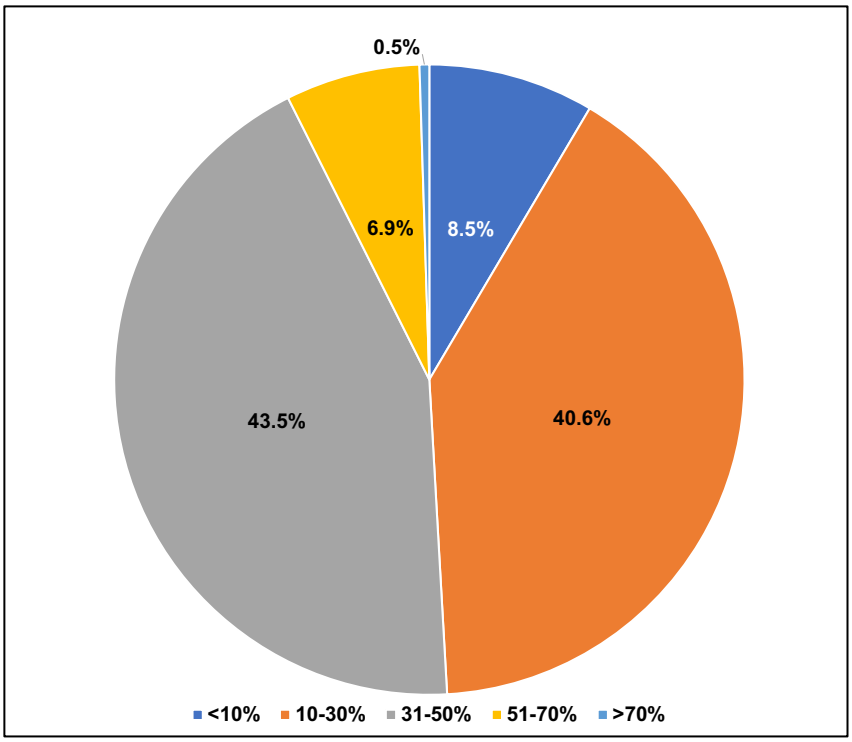


Figure 9. Which is the average percentage of women enrolment in randomized control trials to register new drugs?

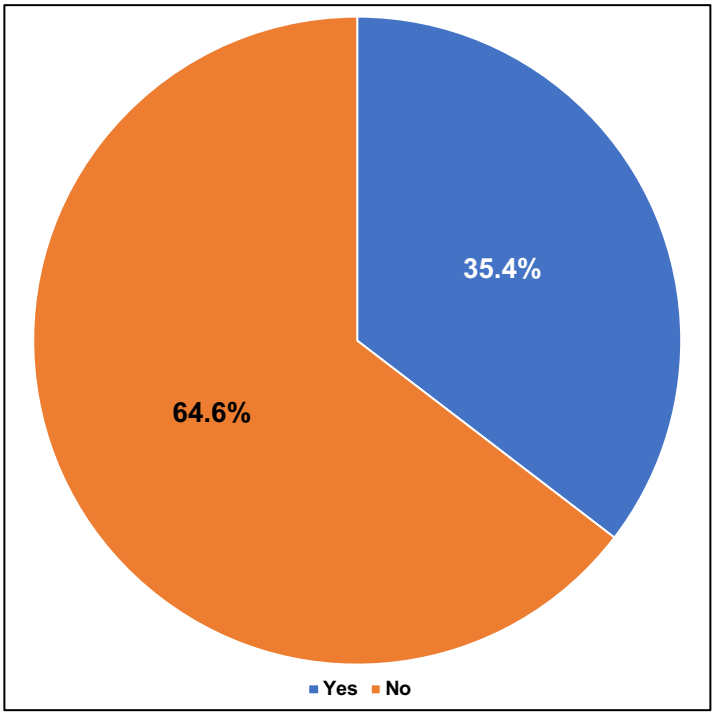


Figure 10. Have you heard of Specific Recommendations for Women in Clinical Guidelines?

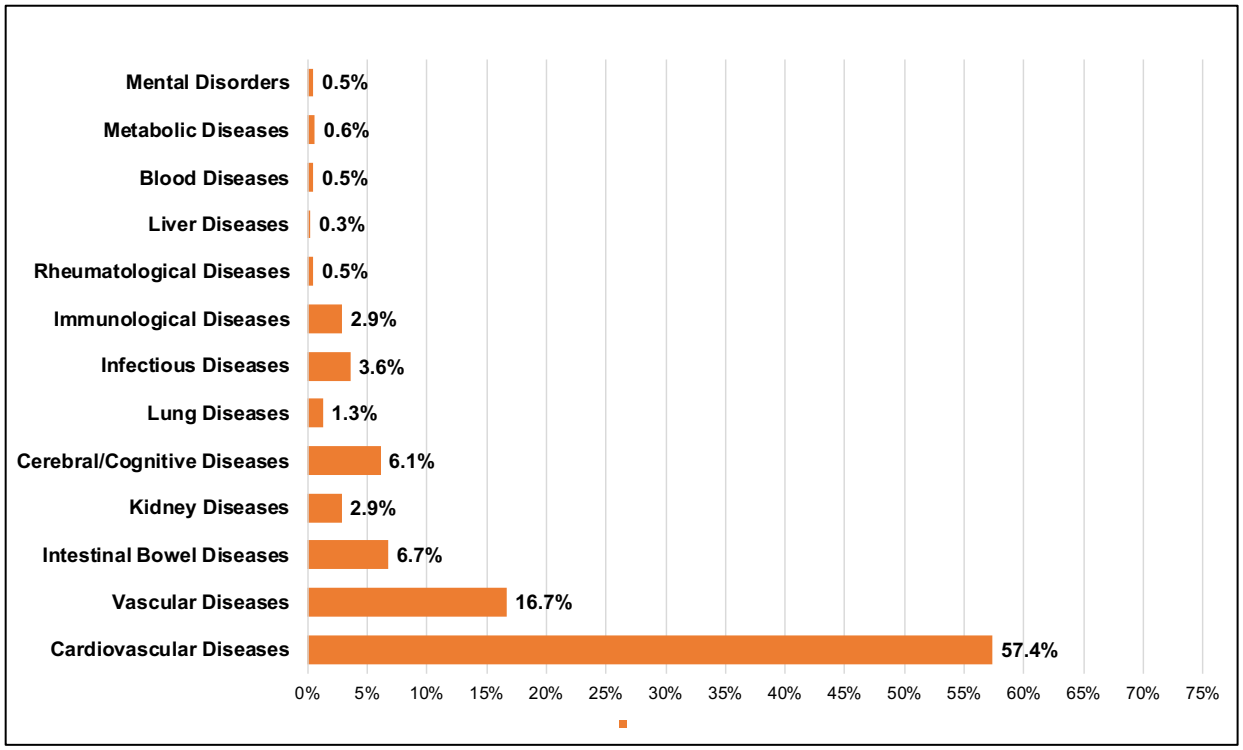


Figure 11. Which areas you are interested in knowing if SGDs exist and influence the clinical management?

Part VI. Precision medicine – impact of gender and biological sex

Chapter 15. Sex, gender and precision medicine.

"Sex, Gender, and Precision Medicine." Biskup E, et al. JAMA Internal Medicine 2020 Aug; 180(8):1128-1129.

Sex and gender should play a central role in everyday personalized medical care. However, in our experiences as early-career physicians and scientists, we observe critical barriers to actualizing this ideal. Sex- and gender-informed approaches to care are founded on community standards appropriately representing biological sex and the complex sociocultural construct of gender. Even though more research is available to provide sex-specific evidence, significant shortcomings remain in effectively implementing sex-informed care. Regarding gender, a community standard could help drive basic and clinical science education for learners, clinical and translational or health services research, and ultimately, the delivery of evidence-based gender-sensitive medical care. But such a standard does not yet exist.

Young internists, like us, are dedicated to the study of sex- and gender-sensitive medicine. In work done by the European Federation of Internal Medicine's (EFIM) IMAGINE working group (Internal Medicine and Assessment of Gender differences in Europe) (EB, VR), we are aware that in general internal medicine, there may be a less broad portfolio of sex- and gender-specific medical knowledge and skill than other medical specialties. Additionally, context matters as in some languages, sex and gender are translated identically (e.g. *geschlecht* in German). We believe that undergraduate, graduate, and continuing medical education need enhancements to account for diverse patient populations with respect to sex and gender.

Because of the lack of standard methods to measure gender, research and healthcare delivery may be significantly hampered when caring for gender-diverse individuals. Fortunately, international funding agencies in Europe, Canada, and the United States are increasingly issuing grant calls aimed at incentivizing sex- and gender-informed research. Without these data, we face a bottleneck in advancing basic, translational, and clinical scientific knowledge about the intersectionality of sex and gender with other aspects of human health. There is an abundance of literature that identifies shortcomings of clinical guidelines as they apply to minorities due to exclusion criteria or selective recruitment; this applies also to sex and gender-diverse individuals, who, if not represented in the data, do not benefit from the latest scientific research.

Learning to practice medicine with attention to individual diversity is an essential competency of medical profession. As we envision our long careers ahead, we "*imagine*" being able to deliver to patients -- of all sexes and genders -- personalized, high-quality care as developing scientific knowledge guides increasingly sex- and gender-informed approaches to care.

Chapter 16. Gender and biological sex dimensions –impact on medical oncology field.

"Gender balance at oncology conferences in China." Biskup E, et al. The Lancet Oncology. 2020;21(9):1138-40.

Introduction

Recent data demonstrate that women physicians are less often speakers and organizers at major medical conferences. Most studies examining gender disparities in speakers at various conferences come from North America and Europe. For many specialties, female speakers make up 15-20% of conference speakers, with surgical specialties frequently having the lowest female representation. In oncology, the European Society for Medical Oncology (ESMO) reported an increase of female speakers across international oncology conferences from approximately 25% to 33% between 2000 and 2015. To address such disparities, these studies in North America and Europe suggest that greater female membership on scientific conference planning committees is correlated with an increased proportion of women physician speakers. However, much less is known about such disparities in the Asian continent, specifically in China. To our knowledge, there are no such studies done to examine sex distribution among medical conference speakers in China, especially at oncological and breast cancer conferences.

In 2018, China accounted for 4.3 million, or 24%, of new cancer diagnoses and 2.9 million, or 30%, of cancer mortality globally. Breast cancer was the most common cause of cancer deaths among females worldwide and is the second most common cause of death due to a malignant tumor among females in China.

In China, there are four major oncology conferences: the Chinese Society of Clinical Oncology (CSCO) hosts the two largest annual oncology conferences in Asia, the CSCO meeting (>30,000 participants) and the Breast Oncology Summit (BRCA-CSCO, >3,000 participants); China Anti-Cancer Association (CACA) hosts two events: the annual Chinese Symposium on Medical Oncology (CSMO, >16,000 participants) and Chinese Conference on Oncology (CCO, >14,000 participants).

The aim of this study was to investigate the gender distribution of speakers and scientific committee (SC) members at these four main oncological conferences in China.

Methods

Data collection

We performed a retrospective audit of speakers and scientific committee (SC) members at four oncology conferences in China that took place from 2009 to 2019. Information was obtained from Chinese-language scientific programs available on the respective event websites (www.csco.org.cn; www.csco-bcf.com; http://meeting.csco.org.cn/19/cn; www.csmo.org).

Since different events consisted of various presentation formats and types, we defined speakers as keynote or plenary speakers, moderators or panelists, and podium presenters who were listed as part of the main scientific program. Poster presentations were excluded. Data was not publicly available regarding the total number of submissions and the gender of submitters. One author (XZ) tallied speaker gender for each event for each year, verifying each listed speaker's name on the program with their gender if discoverable through publicly searchable online databases of Chinese academics. These include China National Knowledge Infrastructure and Wanfang and Chinese Science Citation Database. If unavailable in these databases, general internet search was performed. If gender could not be verified, then these speakers were classified as unverified gender.

SC members were defined as chairs or members of program committees for each event who were responsible for the conference scientific program and ultimately approved the final roster of speakers. For CSCO and CACA, SC members are chosen by the respective society's presidents each year; also, speakers or SC members are not required to be members of the society for whom they are planning the conference.

Statistical analysis

Descriptive statistics on speaker gender were performed. Cochran-Armitage trend test assessed the trends in the proportion of speakers and SC members of each gender in relation to the total speakers and SC members, respectively, over time (reported as P for trend). Spearman rank correlation coefficient assessed the total number of speakers and SC members of each gender over the years from 2009 to 2019. P-values of < 0.05 were considered to indicate statistical significance. Analyses were conducted with *R* (version 3.3.2).

Results

The number and proportion of speakers of each gender per year for each of the four conferences from 2009 to 2019 are summarized in Table 1 and Figure 1 (webappendix, page 1 and 2). Twelve CSCO and three BRCA-CSCO speakers' gender could not be verified and were excluded from further analysis.

Over the past ten years, the proportion of CSCO female speakers and SC members increased from 83 (27.8%) female speakers in 2010 and 191 (34.3%) in 2019 (P=0.012); there were 15 (21.4%) female SC members in 2010 and 49 (28.3%) in 2019 (P=0.022) . Similarly, the proportion of BRCA-CSCO female speakers increased from 4 (17.4%) in 2010 to 14 (40.0%) in 2019 (P=0.001). SC members increased from 6 (16.7%) to 24 (40.7%) in 2019 (P=0.006).

The proportion of CCO female speakers increased from 68 (25.37%) in 2010 to 369 (35.8%) in 2019 (P<0.01). SC members increased from 2 (4.65%) in 2010 to 14 (17.7%) in 2019 (P=0.007). The proportion of CSMO female speakers increased from 23 (26.7%) in 2010 to 42 (47.7%) in 2019 (P<0.01). SC members increased from 17 (26.6%) in 2010 to 39 (31.2%) in 2019 (P=0.238). Of trend tests performed for all conferences, only the proportion of CSMO female SC members increased without statistical significance.

Spearman rank correlation demonstrated a positive correlation between the number of CSCO female speakers and year (R2=0.750, 95%CI: 0.279-0.964, P=0.006); the positive correlation was stronger for BRCA-CSCO female SC members and year (R2=0.910, 95%CI: 0.758-0.995, P < 0.001); CCO female speakers and year (R2=0.903, 95%CI: 0.854-1, P=0.007) and CCO female SC members and year (R2=0.910, 95%CI: 0.527-1, P=0.006); and CSMO female SC members and year (R2=0.954, 95%CI: 0.764-1, P<0.001).

Spearman rank correlation demonstrated a strong positive correlation between the number of CSCO female speakers and year (R^2=0.697, P=0.025); BRCA-CSCO SC members and year (R^2=0.818, P=0.004); CCO female speakers and year (R^2=0.903, P<0.01) and CCO SC members and year (R^2=0.829, P<0.01); and CSMO SC members and year (R^2=0.954, P<0.01). Data available upon request.

Discussion

Findings demonstrate that the proportion of female conference speakers and SC members of four oncology conferences in China has been increasing between 2009 and 2019. This suggests that female physicians are increasingly well-represented in these conference settings. Similar to previous findings, the proportions of female speakers and female SC members appeared to both increase over time.

A previous study showed that women were 56% of graduating medical students in China. In 2019, among all 3,607,000 physicians in China, 1,955,000 were men and 1,652,000 (45.8%) women. In oncology, women were 55% of breast cancer medical oncologists and 35% of breast cancer surgical oncologists in China in 2019. From the professional medical societies' websites, women were 38.6% of CSCO members and 49.8% of CACA members in 2019. Publicly available membership data indicate that CSCO has more surgical oncology members than CACA. This suggests that CSMO, a medical oncology conference hosted by CACA, may appropriately have the highest representation of female speakers (47.7%) as of 2019, compared to the three other Chinese oncology conferences. This may also reflect the higher proportion of female medical oncologists in the workforce as well. Nevertheless, the overall female speaker representation across all four conferences still appears to fall short by comparison to the proportion of female members. Possible differences could be explained by medical oncology vs. surgical oncology or other oncologic subspecialties, however, public data on specialties of each speaker were not collected.

The findings are potentially promising. Over the 10-year period of data collected, all conferences demonstrated an increase in the total number of speakers. For example, in CSCO, the total number of speakers almost doubled during this time. Because the proportion of female speakers also increased, the increase in absolute number of female speakers is more pronounced. Our results further, suggests that female medical oncology specialists, including breast oncologists, may be better retained in the physician training and workforce pipeline. However, it is unclear which is the primary driver. It may be that representation of female conference speakers and scientific committee members drives pipeline retention for female medical oncologists or vice versa. Further investigation is needed to investigate contributors to the observed pattern. This

includes examination of local cultural or political factors that could influence work-life integration and the leadership pipeline for Chinese women physicians.

Limitations of this study include the lack of clear speaker and committee eligibility or selection criteria, meaning that it is unknown how many eligible female speakers are in the denominator of all potentially eligible speakers. This may introduce bias that cannot be controlled for in the present observational approach. Additionally, while workforce data on gender distribution are available in more recent years, the lack of data on earlier periods limits interpretation of trends over longer time periods. Nevertheless, the current analyses are a promising step towards studying and addressing gender inequity for women physicians in China at medical conferences. Gender equity in the global physician workforce remains an unreached goal that would benefit patients and physicians.

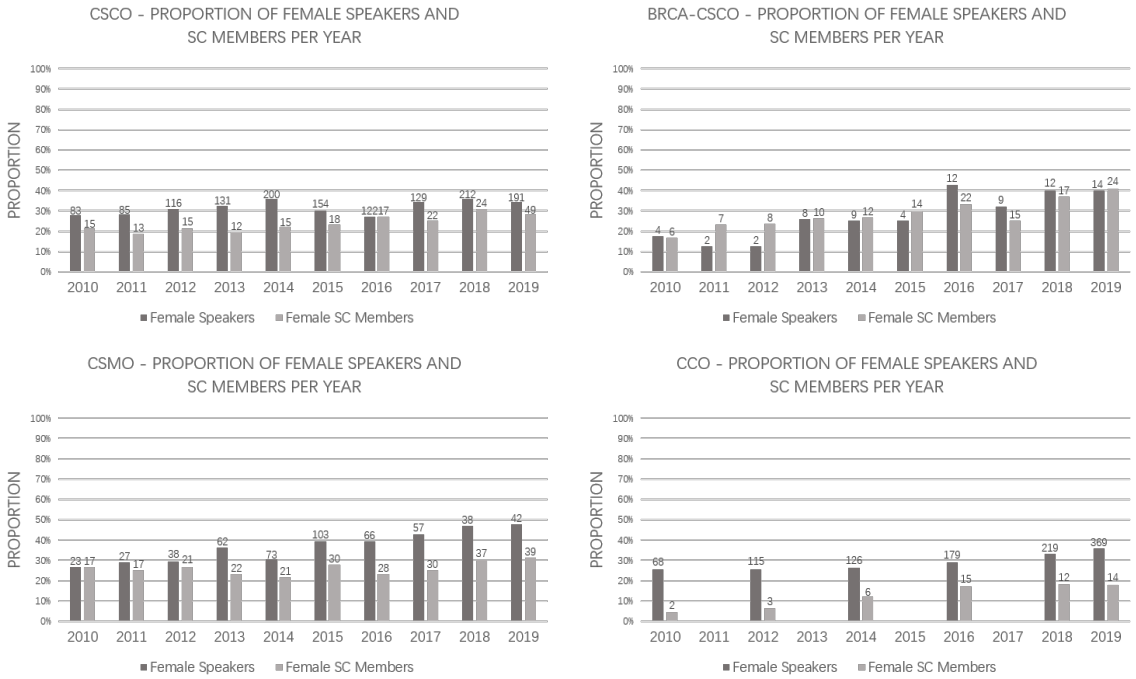


Figure 1. Number and proportion of female speakers and scientific committee (SC) members at each oncology conference in China by year. CSCO = Chinese Society of Clinical Oncology; BRCA-CSCO = Breast Oncology Summit;

Chapter 17. Biological sex and gender in the prisma of COVID-19 pandemics.

Towards precision medicine: inclusion of sex and gender aspects in COVID19 clinical studies – acting now before it's too late. A joint call for action. in collaboration with WHO and UN, Published in The Journal of clinical investigation 2020 Jul;130(7):3350-3352.

Off the back burner: diverse and gender-inclusive decision-making for COVID-19 response and recovery. Bischof E, et al. BMJ Global Health. 2020;5(5):e002595.

The COVID-19 global pandemic is accelerating investigations for effective vaccines and repurposable validated therapeutics. Current data analyses strongly suggest that the disease mostly affects the elderly population and patients with pre-existing conditions. Considerably less attention has been drawn towards the sex distribution of case fatalities, which is increasingly showing disparities in mortality rates that varies geospatially, and along socioeconomic factors. Reported death estimates by sex vary greatly across contexts and population groups and may change over time. In addition, social factors, such as testing and reporting bias in females, or differences in exposure due to behavioral and risk differences, may play a role, e.g., due to comorbidities such as diabetes or differences in societal and gender norms. While the observed male dominance in COVID-19 prevalence and mortality across most countries and cultures may suggest a role for biological differences, the potential long-term impact of gender-related factors on mortality, especially in diverse socioeconomic contextS, cannot be underestimated.

The role of immunological differences between females and males in the responses to SARS-CoV-2 infection appears to be justified. There is ample evidence that antiviral immunity differs between the sexes. These are caused by e.g., sex steroid hormone signaling (i.e., testosterone, estrogens, and progesterone), genetics (e.g., immune function genes that escape X inactivation), and sex-specific composition of the microbiome. Sex differences in immunosenescence and immune function not only impact immunity to viruses, but to vaccines and immunotherapies, as well. In the context of SARS-CoV-2, these differences could impact susceptibility and initial response to the virus as well as choice of acute and long-term therapy of the COVID-19 pathology. In current and future trials for COVID-19, sex as a biological variable should be factored in and understood, along with the wider gendered implications of the COVID-19 crises, with the broader concept of how biological factors intersect with gendered differences in exposure, transmission, and socio-economic means. Consequently, the pandemic may not just lead to differences in disease susceptibility and manifestation between men, women, and people with non-binary identities, but also exacerbate unequal access to treatment and long-term vulnerabilities.

Given their non-negligible impact on health, sex and gender dimensions, along with other socio-economic stratifiers, need to inform the design, conduct, analysis, and reporting of current and forthcoming trials. Moving beyond sex-disaggregated data collection and including variables such as disability, age, ethnicity, migration status, socioeconomic status, or geographic location, will contribute to ensure health benefits from clinical trials for all. To better understand and respond to the burden posed by COVID-19, both on health systems and different segments of

human populations, gender dimensions must be recognized as an intersecting component of wider structural inequalities. Moreover, the COVID-19 pandemic is exposing, most acutely, the wider social inequalities that are based on gendered social, cultural, and economic faultiness, whether it is leaving a majority of frontline workers (in many contexts mostly women) without PPE, the disproportionate burden of unpaid care on women, or gender-based violence perpetuated within the household, apart from the economic devastations experienced by the poor and women in the poorest quintiles.

Equity in clinical trials starts with the consideration of both sex and gender dimensions in studies on novel and repurposed drugs. Biomedical AI-researchers can assist in this effort to reconceptualize the human subgroups included for analysis, emphasizing the rigorous justification of exclusion and avoiding assumptions that may have serious implications in terms of generalizability of outcomes. Ignoring aspects of sex and gender in data collection and analysis in clinical trials has had detrimental consequences in the past. Eight out of ten drugs withdrawn from the US market in the late 1990s had significantly more side effects in women than men, including fatal torsade-de-pointe after excessive QT interval prolongation. The, yet to be accurately tested, proposed therapeutic regimen of hydroxychloroquine and azithromycin for COVID-19 includes two QT-prolonging agents. Next to potential sex differences in side effects, gender-related aspects have to be considered. For example, despite the disproportionately high mortality of Ebola viral disease (EVD) among pregnant women, the rVSV-ZEBOV vaccine clinical trials excluded pregnant women. This impacted access to critical life-saving interventions during the subsequent Tenth EVD epidemic in DRC, when—due to lack of evidence because of the beforementioned exclusions—pregnant and lactating women did not partake in ring vaccination campaigns, until the decision was reversed 10 months later. This not only led to unnecessary mortality of this vulnerable group, but severely impacted women's right to decide on research participation and community trust in the intervention, just as did the approval of Truvada solely for cisgender males and transgender women.

The inclusion of sex and gender aspects in drug development and clinical trials is essential, not just for a thorough understanding of efficacy and safety aspects of drugs, but also to ensure there is equity in the distribution of innovation and discovery benefits of COVID-19 therapeutics and vaccines. A group of clinicians, scientists and gender specialists working on global health, sex and gender research and human rights are thus calling for action towards the inclusion of sex and gender, and other socially relevant variables, into the methodology of COVID-19-related trials. Such an approach should become a universal and manifest part of future clinical studies, to allow more personalized patient care and contribute to universal health coverage.

Part VII. Basic research cardiology

Chapter 18. Myocardial Function and Microcirculation

Effects of Methylprednisolone on Myocardial Function and Microcirculation in Post-resuscitation: A Rat Model. Bischof E, et al. Front Cardiovasc Med. 2022;9.

Abstract

Background: Previous studies have demonstrated that inflammation and impaired microcirculation are key factors in post-resuscitation syndromes. Here, we investigated whether methylprednisolone (MP) could improve myocardial function and microcirculation by suppressing the systemic inflammatory response following cardiopulmonary resuscitation in a rat model of cardiac arrest (CA). **Methods:** Sprague-Dawley rats were randomly assigned to 1) sham, 2) control, and 3) drug groups. Ventricular fibrillation was induced and then followed by cardiopulmonary resuscitation (CPR). The rats were infused with either MP or vehicle at the start of CPR. Myocardial function and microcirculation were assessed at baseline and after the restoration of spontaneous circulation. Blood samples were drawn at baseline and 60-min post-resuscitation to assess serum cytokine (TNF-α, IL-1β, and IL-6) levels. **Results:** Myocardial function (estimated by the ejection fraction (EF), myocardial performance index (MPI), and cardiac output (CO)) improved post-ROSC in the MP group compared with those in the control group ($p < 0.05$). MP decreased the levels of the aforementioned pro-inflammatory cytokines and alleviated cerebral, sublingual, and intestinal microcirculation compared with the control ($p < 0.05$). A negative correlation emerged between the cytokine profile and microcirculatory blood flow. **Conclusion:** MP treatment reduced post-resuscitation myocardial dysfunction, inhibited pro-inflammatory cytokines, and improved microcirculation in the initial recovery phase in a CA and resuscitation animal model. Therefore, MP could be a potential clinical target for cardiac arrest patients in the early phase after cardiopulmonary resuscitation to alleviate myocardial dysfunction and improve prognosis.

Introduction

Cardiac arrest (CA) is a highly fatal medical emergency. Despite established guidelines, even an early intervention yields an approximately 50% success rate of resuscitation, of which only 0.8%-20.1% of patients reach a hospital discharge in the United States. Myocardial dysfunction is the primary cause of early death in post-resuscitation, mostly caused by ischemia-reperfusion injury, stimulated inflammatory cascades, and elevated circulating catecholamines. Microcirculatory blood flow is crucial for the recovery of vital organs following ischemia/reperfusion injury following CA. Our previous research revealed a close relationship between the severity of microcirculatory dysfunction and cardiopulmonary resuscitation (CPR) outcomes in a CA rat model.

Methylprednisolone (MP) is a non-halogenated corticosteroid with a methyl group at C6, which augments its anti-inflammatory properties. Several preclinical studies have found that MP attenuates macrophage and leukocyte activation, while decreasing the synthesis of inflammatory cytokines. Endothelial damage is associated with systemic inflammation following myocardial

injury. Post-resuscitation therapeutic strategies are aiming at to attenuating the inflammatory response following a successful CPR. Meanwhile, current research has indicated an enhanced survival rate in CA patients after administration of combined adrenaline, vasopressin, and MP. However, the effects of MP as a single drug therapy approach for improving cardiac function in CA patients post-CPR are not yet known. In this study, we investigated the cardioprotective effect of MP as a single agent in a post-resuscitation animal model.

Materials and Methods

This work was approved by The Institutional Animal Care and Use Committee at the Virginia Commonwealth University and Guide for the Care and Use of Laboratory Animals (published by the National Institutes of Health) was followed while handling the rodents. All procedures were conducted in accordance with institutional guidelines. Envigo (a single-source breeder) was the source of 6–7-month-old healthy male Sprague-Dawley rats (weight range: 450–550 g) on which ventricular fibrillation was induced.

Animal Preparation

Animals (n = 18) were fasting overnight, with access to water. Pentobarbital (45 mg/kg) was used to anesthetize the animals via intraperitoneal injection, with further administration of 10 mg/kg every hour if necessary to maintain the level of anesthesia. A 14-G cannula set on a blunt needle (Abbocath-T; Abbott Hospital Products Division, North Chicago, IL, USA) with a tip at 145 degrees was used for tracheal intubation. Constant monitoring of end-tidal CO_2 ($ETCO_2$) was performed using a side-stream infrared CO_2 analyzer (Capstar-100; CWE Inc., PA or End-Til IL 200; Instrument Laboratory, Lexington, MA, USA) fitted between the ventilator and tracheal cannula. The pressure in the right atrium was quantified using a PE-50 catheter (Becton-Dickinson, Franklin Lakes, NJ, USA) with high-sensitivity transducers (model 42584-01; Abbott Critical Care Systems, North Chicago, IL, USA) placed into the right atrium through the left external jugular vein. A catheter was placed in the descending aorta through the left femoral artery. Blood temperature was quantified using a 10 cm long and 0.5 mm wide thermocouple microprobe (9030-12-D-34; Columbus Instruments, Columbus, OH) positioned into the left femoral vein.

To induce ventricular fibrillation (VF), a 3-F PE catheter (Model C-PMS-301J; Cook Critical Care, Bloomington, IN, USA) was inserted into the right atrium via the right external jugular vein. A saline solution containing 2.5 IU/mL of bovine crystalline heparin was introduced through the catheter regularly. To maintain the blood temperature (core temperature) at 37 ± 0.5 °C, constant monitoring of a standard lead II ECG was used along with a heating blanket. The peritoneal cavity was exposed via laparotomy via a midline abdominal incision (~ 1.5 cm). Dehydration and body heat loss were minimized by covering the wound with sterile gauze saturated with normal saline solution at 37 °C. A craniotomy was performed, and the right parietal bone was excised without damaging the dura mater (14).

Experimental Procedures and Treatments

The rats were randomly assigned (by a simple randomization method, where the degree of freedom (E) of variance analysis is E =number of all animals-the number of groups) to one of three groups (six animals per group): (1) drug: rats in this group underwent VF, CPR, and received MP treatment; (2) control: rats in this group underwent VF, CPR, and received saline; and (3) sham: animal preparation was the same but without VF induction or CPR.

A baseline measurement was performed 15 min before VF induction, while mechanical ventilation was initiated at 100 breaths per min (frequency), using a volume of 0.60 ml/100 g of body weight (tidal volume) with an inspired O_2 fraction (FiO_2) of 0.21. Electrical induction of VF involved a cumulative current administered to the endocardium of the right ventricle with a 60-Hz voltage and a maximum of 3.5 mA intensity current.

Spontaneous defibrillation was prevented by continuing the current flow for 3 min. Mechanical ventilation was stopped at the start of VF. Initiation of precordial compression (PC) was done 8 min after the starting of VF and was coupled with mechanical ventilation at a frequency of 100 breaths a minute with FiO_2 of 1.0. The PC was kept constant at 200/min and was coordinated with the number of ventilations in order to supply a 2:1 compression/ventilation ratio. The compression and relaxation were equal. The CPP (coronary perfusion pressure) was maintained at 22±2 mmHg while the $ETCO_2$ was 11±2 mmHg after an initial adjustment of the compression depth. Resuscitation consisted of up to 3 four-joule counter shocks. CPR (30s intervals) was followed by a subsequent sequence of up to 3 shocks in the case of failure of ROSC (return of spontaneous circulation). One minute after PC initiation, an injection of 30 mg/kg of MP (8% concentration diluted in 0.375ml/kg NaCl 0,9%) or 0.375ml/kg vehicle (saline 0,9%) into the right atrium was performed over a 10-sec interval. ROSC was considered the maintenance of an average aortic pressure over 50 mmHg for at least 5 min. Once ROSC was achieved, mechanical ventilation was continued using 100% oxygen for the first 60 min, 50% for the next 60 min and 21% afterward. Euthanization was performed by administrating an overdosage of 150 mg/kg pentobarbital via the femoral artery. The injuries induced by the surgery to the viscera, thorax, and abdominal vessels were assessed during necropsy. Supplement Fig. 1 illustrates the experimental protocol of the study.

Quantifying Hemodynamics

A personal computer-based data acquisition system (DATAQ Instruments, Akron, OH) was used to record the pressures inside the aorta and the right atrium, the electrocardiogram, and the $ETCO_2$ for a period of six hours. The CPP was calculated as the difference in real-time between pressures.

Echocardiography Measurements

Myocardial function was quantified by evaluating the ejection fraction (EF), myocardial performance index (MPI), and cardiac output (CO) at baseline and 2, 4, and 6 h post-ROSC using echocardiography (HD 11 XE, Philips Healthcare, Andover, MA, USA) with a 12.5 MHz transducer. Two investigators performed the quantifications individually and were blinded to the study groups.

Microcirculation Evaluation

Cerebral, sublingual, and intestinal microcirculation was monitored at baseline and 1, 3, and 6 h post-ROSC using a 5× optical probe containing side stream dark-field imaging (MicroScan; MicroVision Medical Inc., Amsterdam, Netherlands). A sublingual microcirculation assessment was performed at the base of the tongue. The approach of Qian et al. was applied to measure intestinal microcirculation (16). Brain microcirculation was observed in the right parietal lobe. The microcirculatory flow index (MFI) assessed the blood flow in vessels with a diameter of less than 20 μm. Vascular density was quantified using images, as described by De Backer et al (17). Functional capillary density was estimated using the perfused vessel density (PVD) parameter calculated as the product of the perfused vessel proportion and vessel density. The average values for the three areas were calculated for each animal.

ELISA

To quantify TNF-α, IL-6, and IL-1β serum cytokine levels, blood samples (1 ml) were collected at baseline and 1h after ROSC, followed by immediate centrifugation at 4000 rpm at room temperature for 10 min and stored until analysis. An enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) was used to quantify the cytokines in accordance with the manufacturer's protocol.

Statistical Analysis

Data are presented as mean ± SD or median (interquartile range). All variables were compared using analysis of variance (ANOVA) (parametric test) or the Mann-Whitney U test (nonparametric test). Repeated-measurement analysis of variance was conducted for intragroup alterations of time-based measurements. After obtaining a difference between the groups, we used the Bonferroni test to compare any other two groups. Pearson's correlation coefficient was calculated for linear correlations. Statistical significance was set at a p-value of <0.05.

Results

All 18 rats were successfully resuscitated. The electrical current required to induce VF and CPP during CPR and the number of defibrillations necessary to restore ROSC were comparable among all groups. CPP was 22 ± 2.2 mm Hg and 23.5±2.4 mm Hg in the drug and control group, respectively. The number of defibrillations were 1.3 ± 0.5 and 1.9 ± 1.6 in the drug and control group, respectively.

Effect of MP on Post-Resuscitation Hemodynamics

No evident differences between baseline hemodynamics were observed (Supplementary Table 1). Post-resuscitation, there were no differences in heart rate and $ETCO_2$ between the animals in the drug and control groups. The drug and control groups displayed a reduction in mean arterial pressure (MAP) following resuscitation compared to the baseline and sham groups. However, MP administration caused a significant increase of MAP 5 h and 6 h post-ROSC, which was not observed in the control group .

Effect of MP on Myocardial Function Post-Resuscitation

The three groups displayed no differences in myocardial function (EF, CO, and MPI) at baseline (Supplementary Table 1). There was an evident reduction in all three functions post-resuscitation compared with their baselines in both the drug and control group. However, in MP-treated animals, the severity of the myocardial dysfunction post-resuscitation, especially MPI, was significantly lower compared with control animals at the time points of 2, 4, and 6 h of ROSC (Fig. 1).

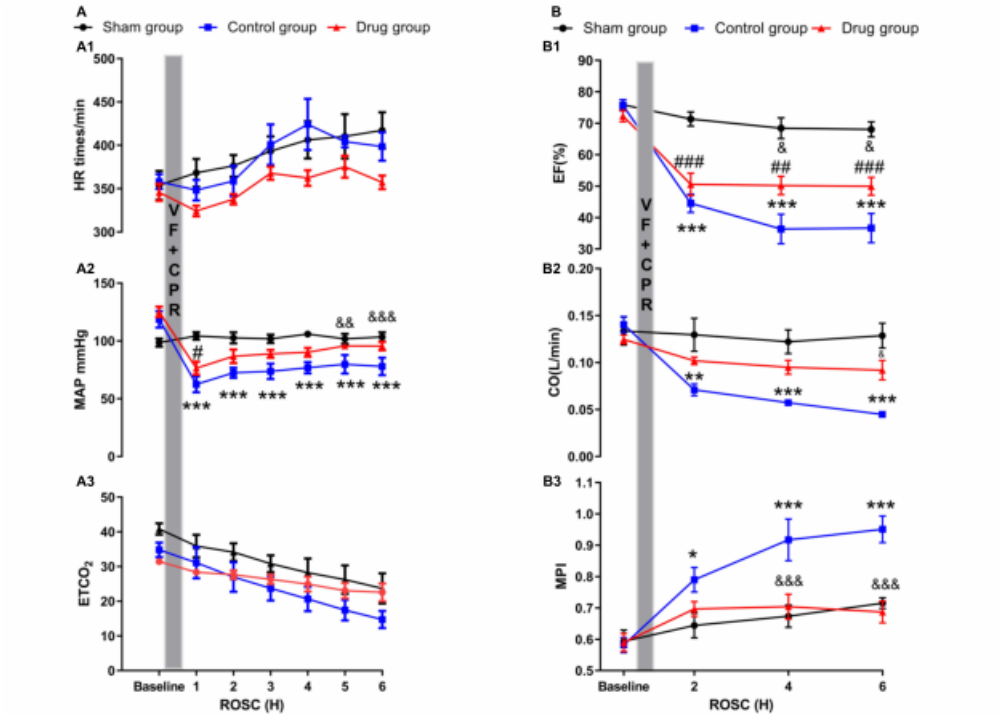


Figure 1. Myocardial Performance and myocardial function. (A1) Heart rate; (A2) Mean arterial pressure; (A3) End-tidal CO_2; (B1) Ejection fraction; (B2) Cardiac output; (B3) Myocardial performance index. HR: heart rate; MAP: mean arterial pressure; $ETCO_2$: end-tidal CO_2; CO, cardiac output; EF: ejection fraction; MPI: myocardial performance index; VF: ventricular fibrillation; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation. Values are presented as mean±SD (n = 6 in each group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ control vs. sham group; $^{\&}$ $p < 0.05$, $^{\&\&}$ $p < 0.01$, $^{\&\&\&}$ $p < 0.001$ drug vs. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, drug vs. sham group.

MP Attenuated System Inflammation

Serum cytokine levels were increased in post-resuscitation state in both drug and control groups compared with sham. However, TNF-α, IL-1β, and IL-6 levels were significantly lower in animals treated with MP at 1 h post-resuscitation as compared to the control animals (Fig. 2).

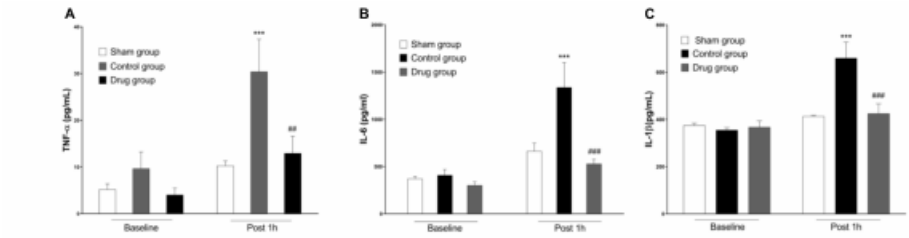


Figure 2. MP effects on post-resuscitation pro-inflammatory cytokines. (A) Plasma levels of TNF-α, (B) Plasma levels of IL-6, (C) Plasma levels of IL-1β. TNF-α: tumor necrosis factor-α; IL-6: interleukin 6; IL-1β: interleukin 1β. Data are presented as mean±SD. ***$p < 0.001$ control vs. sham group; $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, drug vs. control group.

Effect of MP On Post-Resuscitation Microcirculation

Decrease in cerebral, intestinal, and sublingual PVD and MFI were observed at 1, 3, and 6 h post-resuscitation in control animals. However, in MP-treated animals, this reduction was reduced to a non-detectable level, which was not observed in the baseline and sham groups (Fig. 3 and Supplementary Fig. 2). Microcirculatory blood flow was negatively correlated with inflammatory cytokine levels (Table 1).

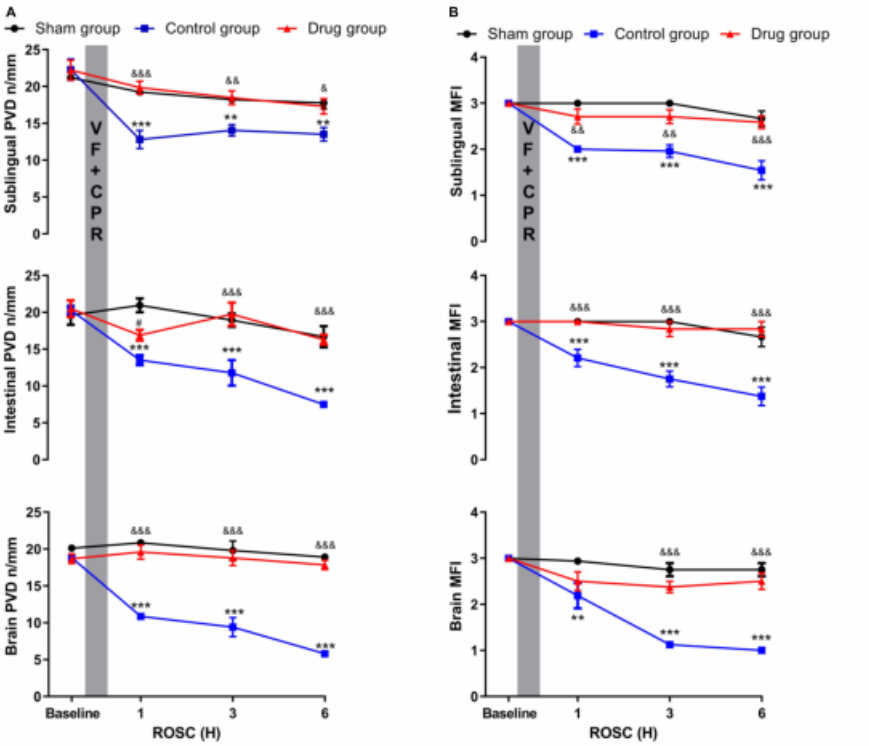


Figure 3. MP effects on post-resuscitation microcirculation. (A) Perfused vessel density, (B) Microcirculatory flow index. PVD: perfused vessel density; MFI: microcirculatory flow index; VF: ventricular fibrillation; CPR: cardiopulmonary resuscitation. Data were presented as mean±SD (n = 6 in each group). **$p < 0.01$, ***$p < 0.001$ control vs. sham group; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, drug vs. control group; $^{\#}p < 0.05$, drug vs. sham group.

	Sublingual		Cerebral		Intestinal	
	PVD	MFI	PVD	MFI	PVD	MFI
Cytokine levels	r	r	r	r	r	r
TNF-α	-0.425*	-0.752*	-0.434#	-0.608*	-0.461*	-0.651*
IL-1β	-0.553*	-0.819*	-0.310	-0.467#	-0.480*	-0.644*
IL-6	-0.630*	-0.699*	-0.532*	-0.621*	-0.498*	-0.801*

PVD, perfused vessel density; MFI, microcirculatory flow index; TNF-α, tumor necrosis factor α; IL-6:Interleukin-6; IL-1β:Interleukin-1β. #p<0.05, *p<0.01.

Table 1. Correlations between microcirculatory parameters and serum cytokine levels.

Discussion

In our study, we observed that the administration of MP improved the objective myocardial function in post-resuscitation model animals who suffered from a global ischemia caused by a cardiac arrest. Reduction of inflammation and improvement of microcirculation-induced ischemia following CPR are plausible reasons for this outcome.

Several clinical studies indicated that high post-resuscitation mortality rates are linked to substantial myocardial dysfunction following successful resuscitation from CA. Similarly, in the present study, the initial post-resuscitation myocardial function was strongly impaired. However, the severity of the dysfunction was significantly reduced in MP-treated rats. In addition to improved myocardial function, we observed a reduction in systemic inflammation and an improvement in microcirculatory blood flow. Clinically, the robust anti-inflammatory effect of MP allows for its use in diseases characterized by inflammation, such as ulcerative colitis, Crohn's disease, and rheumatoid arthritis. In addition, pro-inflammatory cytokines TNF-α and IL-1β engage in the pathogenesis of myocardial dysfunction and cardiomyocyte death in I/R injury has been described. Adrie et al. showed that elevated levels of circulating cytokines in the plasma, along with the aberrant cytokine balance found in CPR patients is similar to the immunological disturbances found in patients with sepsis. Furthermore, a recent multicenter observational study found that higher levels of IL-1Ra, IL-6, IL-8, and IL-10 were present in non-survivors and survivors with poor functional outcomes. Moreover, an elevated IL-6 level following ROSC has been linked to worse outcomes. In the present study, we demonstrated that systemic inflammatory cytokines, such as TNF-α, IL-6 and IL-1β measured in the post-resuscitation state, were significantly reduced by MP in a rat model of CA post-CPR. This result indicates that the reduction in pro-inflammatory cytokines caused by MP might be a possible mechanism for the increased post-resuscitation myocardial function observed in our study.

We further demonstrated that MP improved post-resuscitation microcirculation. Microcirculatory dysfunction was minimal in MP-treated animals, with the baseline value being almost reached at 1 h post-ROSC and maintained during the 6-hour observation post-resuscitation period. In MP-treated animals, an increase in microcirculatory blood flow was negatively correlated with a decrease in inflammatory cytokine levels. The close association between the severity of microcirculatory dysfunction and vital organ function post-resuscitation has received significant

attention among cardiology and intensive medicine science. Our previous work in animals also showed that microcirculatory flow and serum cytokine levels are closely linked. Improvement in microcirculation after myocardial ischemia/reperfusion in STZ-induced diabetic animals treated with MP via TLR4/NF-κB signaling inhibition has been reported. We also found that administration of MP after CPR increased the MAP. Increased post-resuscitation microcirculation indicates enhanced organ perfusion. Overall, our results show that post-resuscitation "sepsis-like" syndrome triggered by whole-body I/R is most likely the underlying mechanism of microcirculatory dysfunction after successful resuscitation. Moreover, microcirculatory and myocardial functions can be increased in the post-resuscitation state by administration of anti-inflammatory drugs.

Although there are clinical trials that show that hospitalized cardiac arrest patients receiving vasopressin plus methylprednisolone or epinephrine at resuscitation do not have an improved 30-day survival, several randomized, double-blind trials, published in 2009 and 2013, Mentzelopoulos et. al. and Andersen et. al. compared the addition of vasopressin and glucocorticoids during cardiac arrest with placebo, showing a large improvement in outcomes. Moreover, in this study, we have implemented a ventricular fibrillation animal model.

Since a single dose of MP was used in the study, further protocols with varying MP administration patterns for effect comparison are pending. Furthermore, a limitation occurred since the study focused on measuring of the pro-inflammatory cytokines at 1 h post-resuscitation, there are no conclusions to be drawn regarding their changes in further post-resuscitation recovery. Additionally, intergroup survival analyses were not performed.

Another limitation is of methodological nature: MFI is based on a semiquantitative scoring system to assess the magnitude of microvascular perfusion and is thus considered to be a subjective parameter and was not recommended in the second consensus. Furthermore, the Microvision camera we have used is designed for analyzing sublingual microcirculation. While data on any other microvascular beds are limited, several studies have investigated intestinal and brain microcirculation by the Sidestream Dark-Field (SDF) imaging method. This technique has been found as highly promising for the visualization and assessment of bowel microcirculation in a study performed on patients during gastrointestinal surgery.
Sidestream dark field imaging is a very promising technique for bowel microcirculatory visualization and assessment.

In conclusion, the present study showed that MP reduced the severity of post-resuscitation myocardial dysfunction by targeting pro-inflammatory cytokines and improving microcirculation. Therefore, our study suggests that MP could be a potential therapeutic for ROSC patients after a cardiac arrest, especially when administered at an early phase after CPR, in order to alleviate myocardial dysfunction and improve prognosis.

Chapter 19. IL-6 inhibitors in post-infarction cardiac injury and ischemic myocardial remodeling

"IL-6 inhibitors effectively reverse post-infarction cardiac injury and ischemic myocardial remodeling via the TGF-β1/Smad3 signaling pathway." Biskup E, et al. Exp Ther Med. 2022 Jul 18;24(3):576.

Introduction

Recent data increasingly underline the role of Interleukin 6 (IL-6) in the etiology and progress of heart failure due to post-myocardial injury. These insights are of uttermost experience since the immediate and long-term consequence for patients include cardiac insufficiency and heart failure. Cardiac remodelling processes are highly differing in their extent depending in individuals and often limited. While it is still not fully understood to what extent the inflammatory response and increased oxidative stress are involved in myocardial necrosis it is undeniable that both the myocardial infarction (MI) itself, as well as the golden standard percutaneous *coronary* intervention (PCI) are causing IL-6 increase in the cardiac tissue, which is thus largely at risk of permanent damage.

At present, studies on ventricular remodeling indicate that the ventricular remodeling mechanism mainly includes changes in the nervous and endocrine systems, as well as in cytokines constellation, various cellular signal transduction pathways and mutations pattern. The TGFβ1-Smad3-MMP2/9 signaling pathway is considered to be a common pathway for tissue fibrosis. As a signal transduction factor of TGF-β1, Smad protein is also involved in cardiac development, cell proliferation, growth, and apoptosis. It can interact with other transcription factors and regulate its activity through cytokines.

The severity of myocardial injury determines the occurrence and development of ventricular remodeling, and rational drug treatment is crucial to improve this pathological process. Nowadays, the therapeutic drugs for ventricular remodeling mainly include: β-blockers, angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type receptor antagonists (ARB), often used in combination. More effective inhibition of ventricular remodeling. At present, widely used ACEIs include benazepril, captopril, ramipril, etc. In addition, drugs that inhibit ventricular remodeling also have calcium channel blockers (CCBs), which can selectively block Ca2+. Through the Ca2+ channel on the cell membrane, which reduces the intracellular Ca2+ concentration, which will help reverse the myocardial remodeling phenomenon; and statins, such as simvastatin can effectively inhibit the hypertrophy and fibrosis of cardiomyocytes, simvastatin can also inhibit the synthesis of RhoA and Rho-GTPase in cardiomyocytes, reduce the formation of fibroblasts, thereby preventing tissue fibrosis and preventing cardiac hypertrophy.

Cardiovascular disease is predominantly found in patients who are biologically aged, either based on the chronology, or premature aging, such as due to autoimmune, chronic inflammatory or musculoskeletal conditions. Almost a quarter of all heart attack patients are over 75 years old. A significant increase is expected, as we are facing both a silver tsunami, as well as a large number of younger patients with cardiac damage due to comorbidities or detrimental lifestyle

habits. The incidence of myocardial infarction, recurrences and MACE, as well as the need for subsequent treatment of heart failure in older patients is much higher, while the prognosis is poorer than in younger individuals, with a 20-25% in-hospital mortality rate. Overall, the lethality increases almost exponentially with increasing age: patients in the eighth decade of life have a mortality rate of 17%, patients in the ninth decade of life of 33%.Most of the deaths are based on the depleted "biological reserve". Therefore, preservation of the cardiac muscle after a stressor, is of uttermost importance. We postulate that targeting IL-6 can prevent myocytal senescence, thus allowing repair, regeneration and maintenance of the cardiac tissue.

Since most patients nowadays are multimorbid and elderly, a combination therapy is often complicated and preferably to be avoided. More potent agents are urgently needed. There is an unmet need of identifying new pathophysiological pattern that could be used as therapeutic targets. Thus, we explored whether the use of IL-6 receptor inhibitors can be considered as a candidate to contribute towards cardiac function restoration, myocardial remodeling as seen by the myocardial infarction size and collagen content reduction, the effect on myocardial tissue inflammation, specifically investigating the impact on the IL-6/TGF-β 1-MMP signaling pathway.

1. Materials and methods

1.1 Animal model

Male Sprague-Dawley (SD) rats aged 5 months (Tongji University Laboratory Animal Center) were housed in certificated SPF grade facility with individually ventilated cages: temperature 20-24℃, relative humidity 30%-70%, and 12 hours light-dark cycles (8 AM to 8 PM). All experiments were performed complying with the Guidelines of Animal Experiments from the Ethics Committee of Tongji University (Shanghai, China).

Myocardial infarction model was established in rats by ligation of left anterior descending coronary artery (LAD). SD rats were first fixed on the operating table and anesthetized with intraperitoneal injection of sodium pentobarbital (formulated as 2% solution at a dose of 35 mg/kg). The skin was cut along the left iliac crest and the sternal xiphoid line, the muscles and the pericardium separated and the LAD ligated at a depth of 0.3-0.5 mm under aseptic conditions, without other injuries (intentional or unintentional) on the LAD. The V2 lead electrocardiogram was done postoperatively to observe whether it showed ST-segment elevation and gradual pathological Q wave formation. At the same time, the myocardial infarction model was validated by observing the local myocardial color paleness and by echocardiography to demonstrate the local myocardial segmental motility disturbances. This model was assessed as successfully established AMI-rat standard. Only those rats that fulfilled all standard requirements were included.

1.2 Animal grouping

Rats were randomly divided into four groups: 1. normal control group without any treatment - the N group, n=10; 2. LAD ligation with injection of normal saline - the LINS group, n=20; 3. LAD ligation with injection of IL-6 recombinant protein (PHC0066, Thermo Fisher Scientific) –

the LIIL group, n=20; 4. LAD ligation with injection of IL-6 receptor inhibitor (A2012, Selleck) group - the LIILR group, n=20.

1.3 Animal treatment in specific groups

In the LINS group, the LAD was ligated. After the successful preparation of the myocardial infarction model, the AMI area was divided into four points and injected with 20ul of physiological NaCl each (a total of 80ul) at a distance of 1-2 cm. In the LIIL group and LIILR group, IL-6 recombinant protein (1.8 mg/kg)or IL-6 receptor inhibitor (50 mg/kg), respectively, were accordingly injected into the 4 points in the myocardial infarction area.

1.4 Animal survival rate observation

All animals were returned to the cages for feeding and further observation. Mortality in each group was documented daily.

1.5 Cardiac function test

Echocardiography was performed at 1week and 4weeks after the surgery to determine the cardiac function using the Sequoia C256 ultrasound system's (Acuson Co.) linear array probe at a frequency of 8 MHz and a detection depth of 2 cm. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital. The rat chest hair was scraped off, the rat fixed on the back and connected to the V2 lead electrocardiogram. The probe was placed on the rat's chest and the two-dimensional ultrasound showed the level of the short axis of the fundus near the papillary muscle. Then M-mode ultrasound was used to determine the left ventricular end-diastolic and end-systolic diameter (LVEDD, LVESD) of each group. Left ventricular fractional shortening (LVFS) was calculated using the Teichholtz formula and left ventricular ejection fraction (LVEF) values were calculated to assess the cardiac function. Each set of data illustrated the average of three consecutive cardiac cycles.

1.6 Histopathologic studies

After rats were anesthetized with an excess of pentobarbital, their hearts were removed by a thoracotomy and cut along the ligature line to obtain the largest cross-sectional area of the left ventricle. Sections were used for Masson staining, HE staining and immunohistochemistry, while the remains were used to measure the myocardial inflammatory response indicators myeloperoxidase (MPO), reactive oxygen species (ROS), IL-6, TGFβ1 and MMP2/9.

1.7 HE staining

The HE staining was performed for pathological evaluation as previously described. Briefly, after the tissue has been paraffin embedded, sectioned, placed on a slide and dried in an oven, the slide is taken through brief changes of xylene, alcohol and water to hydrate the tissue. The slides are then stained with hematoxylin and rinsed, then stained in eosin. They are then rinsed, taken back through water, alcohol, and xylene, then carefully coverslipped.

1.8 Masson staining

Paraffin sections were prepared from animal myocardial tissue. Masson staining was performed to evaluate the infarct size and degree of fibrosis in the marginal zone. Under the microscope, the cardiomyocytes presented with red cytoplasm, black nucleus and blue-green collagen fibers. Three sections were taken from each animal. The ratio of left ventricular collagen area to left ventricular area was determined as myocardial infarct size under low-power field (2.5×). Local myocardial collagen and inflammatory response were observed under a high-resolution microscope.

1.9 Immunohistochemistry

Animal myocardial tissues were collected immediately when rats were sacrificed, and fixed overnight with 4% neutral formalin and embedded in paraffin. Sections were subjected to immunohistochemistry (IHC) to determine the levels and distributions of IL-6 and TGF-β1. The following primary antibodies were used: rabbit anti-IL-6 (1:100 dilution, ab6672, Abcam, Cambridge, MA), rabbit anti-TGF-β1 (1:100 dilution, ab92486, Abcam, Cambridge, MA). Biotinylated anti-rabbit IgG (1:500 dilution, sc-2004, Santa Cruz Biotechnology, Santa Cruz, CA) antibodies were used as secondary antibodies accordingto the manufacturer's protocol. Images were observed using an Oberver. Z1 microscope.

1.10 Ethics

The study was approved by the Ethics Committee of Tongji University (Shanghai, China), approval reference LL-2021-ZRKX-018. We followed the UK standards of the humane endpoints for the animals protected by the Animals (Scientific Procedures) Act 1986, regulated by Schedule 1 of the Act. Schedule stating that if an animal is anaesthetised for the purposes of a scientific procedure and is not required to recover from the anaesthesia, it can be killed whilst anaesthetised by any method since it is not capable of suffering whilst unconscious.

70 animals were used and euthanized, none found dead before the experimental endpoint and the cause of death for all animals.

2. Statistical analysis

The SPSS software(version 22.0) was used for the analyses. All data were expressed as means ± standard deviation. Comparison of multiple groups was analysed by one-way analysis of variance (ANOVA) with Bonferroni's post hoc test. GraphPad Prism 5.0 (La Jolla, USA) software was used to calculate the significance between groups. Animal survival curves were assessed by Kaplan-Meier method. $P < 0.05$ was considered to have a statistical significance.

3. Results

3.1 Standardization of the acute myocardial infarction rat model

The standard AIM rat model and the postoperative validation points were established. ECG and echocardiography were documented for all rats, as shown in Fig.1

3.2 Survival curve of the animals

There was no animal death in the N group (survival rate was 100%). Compared with the N group, LAD ligation and normal saline injection resulted in a high mortality, with a survival rate of 65%. LAD-ligation and IL-6-injection (LIIL group) had the highest mortality. The LIILR group had a significantly lower mortality rate (survival rate 90 %, significantly higher than in the LIIL group ($p<0.01$). The survival curves of each group are shown in Fig. 2.

3.3 The effect of IL-6 and its receptor inhibitor on cardiac function

Changes in cardiac function were measured using a Doppler ultrasound diagnostic apparatus 1 week and 2 weeks after the LAD ligation induced myocardial infarction (Figure 3). In the LINS group, compared with the N group, the left ventricular short axis fraction LVFS and the left ventricular ejection fraction LVEF showed a gradual decline, with a progressive cardiac chamber expansion. In the LIIL group, injection of IL-6 aggravated these changes. In the LIILR group, IL-6 receptor inhibitors significantly attenuated the ischemia-induced damage to heart function. LVFS and LVEF were higher than in the LINS and LIIL groups, and lower than in the N group. LVESD and LVEDD in the LIILR group were lower than in the LINS and LIIL groups, while higher than in the SHAM group.

3.4 Effects of IL-6 and its receptor inhibitors on myocardial infarction size and collagen content

The myocardial infarction size and collagen content were detected by Masson staining. The myocardial structure of the N group was intact under low magnification. The infarct size was the largest in the IL6 injection group (LIIL group), followed by the ligation group (LINS group). The infarct size of the inhibitor group (LIILR group) was significantly less than the above two groups (as shown in Figure 4).

Under a high power-polarizing microscope, the Masson staining showed that the cardiomyocytes in red and the collagen fibers in blue-green, as shown in Figure 4. There was no obvious collagen in the N group, and the cardiomyocytes were bright red. The myocardial cells in the infarct border area of the rats in the LINS group were disordered, the blue collagen fibers in the interstitium increased, the myocardial bundle was segmented, some collagen fibers were merged, and the vacuolar changes were observed locally. Significant inflammatory response. After treatment with IL-6, there were more blue-green collagen fibers in the myocardial tissue, collagen deposition increased significantly, the inflammatory response was significantly aggravated, and the myocardial structure changed accordingly. Treatment with IL-6 receptor inhibitors inhibited this phenomenon. It can be seen that the inflamed response in the infarct area is weakened, the collagen is reduced, and the residual myocardium is significantly more than the

former two. However, the myocardial structure changes to some extent, and the ventricular wall is relatively weak.

The quantitative analysis in Figure 4 showed that the collagen content in the myocardium of each group was significantly higher than that in the N group. The collagen content increased significantly after injection of IL-6. Injection of IL-6 receptor inhibitor significantly reduced collagen content. These results indicate that IL-6 promotes myocardial collagen deposition and increases fibrosis and inflammatory response.

3.5 The effect of IL-6 and its receptor inhibitor on myocardial tissue inflammation

The myocardial tissue inflammation index (MPO) and oxidative stress index (ROS) were measured in order to quantify the inflammatory reaction in the myocardial tissue. As shown in Fig. 5, the ROS and MPO in the LINS group were significantly higher than in the N group. Injection of IL-6 promoted the ROS and MPO further increase in LIIL group. In the LIILR group (injection of IL-6 receptor inhibitor), the ROS and MPO were significantly lower than in the LIIL and LINS groups.

HE staining was applied to observe the local inflammatory response of myocardial tissue (Figure 6) under high power microscope. The myocardial tissue of the N group showed intact and well-defined myocardial cells, while no obvious inflammatory reaction or myocardial necrosis were observed. In the LINS group, obvious inflammatory reaction and local myocardial hemorrhage and necrosis were visible, with increased fibroblasts. These pathological changes were even more pronounced in the LIIL group, where a large number of inflammatory cells were infiltrated and myocardial cells were necrotic. Injection of IL-6 receptor inhibitors significantly reduced the local inflammatory response and myocardial hemorrhage. This indicates that IL-6 injection significantly increases the myocardial inflammation and fibrosis. On the contrary, the treatment with IL-6 receptor inhibitor decreases both the myocardial fibrosis and the inflammatory response. This suggests that IL-6 promotes fibrosis and inflammation in the infarcted myocardium, and it can be partially reversed with the use of IL-6-inhibitors.

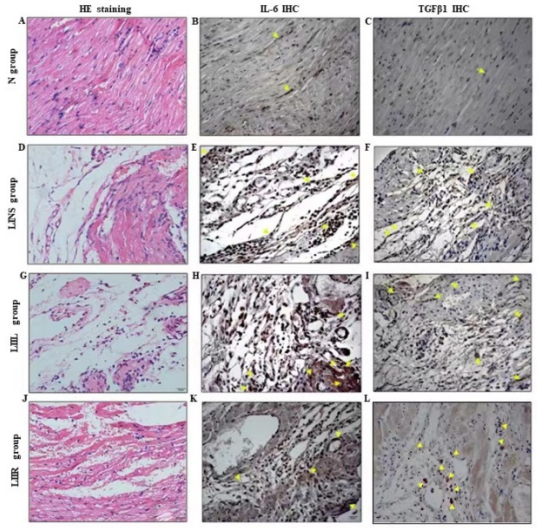


Figure 1. HE staining of myocardial tissue from the N group, the LINS group, the LIIL group and the LIIR group.

To investigate the local expression of the proteins involved in the signaling pathway analyzed, we used immunohistochemical staining and observed the local distribution of IL-6 and TGFβ1 in myocardial tissue. These two factors were mainly expressed in inflammatory regions, vascular endothelium and cardiomyocytes (brown, as shown by the arrows in Fig. 6). Compared with the N group, the number of IL-6 and TGFβ1 positive expression particles in the LINS group was significantly higher. It was further increased in the LIIL group (injected with IL-6), while IL-6R inhibitor injection lead to a significant reduction of the observed expression of IL-6 and TGFβ1 (LIILR group).

3.6 Effects of IL-6 and its receptor inhibitors on IL-6/TGF-β 1-MMP signaling pathway in myocardial tissue fibrosis

To investigate in more details the effect of IL-6/TGF-β1-MMP signaling pathway on the fibrosis process in the infarcted myocardium, first a treatment with IL-6 and its receptor inhibitors was induced, followed by an ELISA analysis of IL-6 and TGFβ1 in the infarcted myocardium. Ligation (induction of the myocardial infarction) increased the expression levels of IL-6, TGF-β1 and MMP-9 in the hearts of rats in the LINS group**.** IL-6, TGF-β1, MMP-2 and MMP-9in the LIIL group were further increased after IL-6 injected. The expression of these factors was significantly reduced after the use of IL-6 receptor inhibitors (Figure 7).

Discussion

In recent years, due to the extensive use of cardiac reperfusion therapy, the survival rate of myocardial infarction patients has improved to a large extent. However, congestive heart failure caused by left ventricular remodeling remains a major clinical problem. The long-term prognosis of these patients is similar to many end-stage malignancies. Left ventricular remodeling is a major factor affecting the prognosis of patients with heart disease. One of the main manifestations is myocardial fibrosis, ventricular dilatation and cardiac insufficiency. Infarct ventricular remodeling occurs as the early (within 72h) or the late remodeling (after 72h), which causes further progressive expansion of early infarct size, left ventricular dilatation, left ventricular wall fibrosis and permanent collagen scar. Especially prolonged remodeling leads to fibrous tissue necrosis and deposition of a large amount of necrotic material, further aggravating the process (vicious cycle). Clinically, this is reflected in cardiac insufficiency.

This study showed that, compared with the normal, control group (N group), a typical left ventricular remodeling occurred in the LINS group at postoperative week 1, with a significant expansion of left ventricular end-diastolic diameter and volume, as well as wall thinning. As a result, the left ventricular pump function was also significantly decreased. After 2 weeks, the changes in the LINS group were aggravated, causing the animal survival rate to further decrease. In addition to observing the structural remodeling of the left ventricle, we also observed the remodeling of myocardial tissue structure in the left ventricle, which showed that the myocardial local inflammatory reaction, myocardial necrosis and collagen fibers deposition was still ongoing. Same processes were observed after IL-6 was injected into the infarct area. IL-6 inhibitor caused a drastic decrease of the remodeling and changes in the intracardiac and wall structure. Overall, these results indicated that IL-6 is an important cytokine in cells, playing a crucial role in the

occurrence and development of left ventricular remodeling. The results also confirmed that the application of IL-6 antagonists, such as Tocilizumb, can largely improve the prognosis of myocardial infarction patients by inhibiting the overall infarct cardiac remodeling.

In our study, we observed that the mortality of the infarcted rats in the LIIL group receiving IL-6 injection was higher than that in the other groups, while the mortality in the LIILR group injected with the IL-6 receptor inhibitor decreased. Cardiac function (LVEF and LVFS) was progressively decreased in rats after acute myocardial infarction. Infusion of IL-6 further impaired cardiac function (LIIL group), while injection of IL-6 receptor antagonist prevented heart function decline of the infarcted rats (LIIL group), suggesting that such an antagonistic mechanism is potentially a therapeutic and preventive target, specifically suitable for post-infarction patients with high IL-6 levels.

The cardiac function was similar between the LINS and LIIL group and the left ventricular remodeling developed in a similar manner. LVEDD and LVESD increased in both post induced infarction (LAD ligation, LINS group), and IL-6 added group (LIIL group). The injection of IL-6 inhibitor inhibited further worsening of both LVEDD and LVESD (LIILR group), indicating that measurable improvements in the overall cardiac function after myocardial infarction is possible with the help of targeted IL-6 inhibition. Furthermore, it could be once again shown that IL-6 plays a crucial role in post-infarction subjects, since IL-6 addition lead to typical post-infarction changes in the clinical outcomes (loss of cardiac function).

Moreover, induced infarction through LAD ligation caused an increase in the inflammation index (increased MPO) and in the oxidative stress index (ROS). Pathological analysis confirmed these changes, demonstrating local obvious inflammatory reaction and collagen formation in the infarcted myocardial area. After injection of IL-6, these pathological changes were aggravated and the infarction area's size expanded. Injection of IL-6R inhibitor significantly reduced local myocardial inflammation and the collagen content. Thus, the physiological changes described above are based on the local histological improvements due to IL-6 inhibition, which allows to suggest that IL-6 inhibitors can be of the uttermost clinical importance in post-MI patients, as they could not only prevent a rapid expansion of the potentially permanently damaged infarction area (thus lost for cardiac function), but also significantly revert the infarction-caused dilapidation of the cardiac tissue. The latter is responsible for most post-infarction syndromes, such as arrhythmia, Dressler's syndrome, cardiac insufficiency etc. All of those significantly decrease patients' quality of life.

As expected, the LAD ligation which induced the myocardial infarction also lead to an increase in the expression of local myocardial inflammatory factors TGFβ1, MMP2 and MMP9. TGF-β1is a main player in the apoptotic cascade. Previous studies reported a close relationship between IL-6 and TGFβ1, where the latter induces phosphorylation of SMAD-2, phosphorylated SMAD-2 and SMAD-4 formation in intestinal epithelial cells.

The complex down-regulates IL-6 signaling. Our study found that the use of IL-6 can significantly up-regulate the expression of TGFβ1 in infarcted hearts, while the injection of IL-6 receptor inhibitor can significantly reduce its expression, suggesting that TGFβ1 may be involved as a key gene downstream of IL-6.

Interestingly, the injection of IL-6 further increased their level, while addition of IL-6R inhibitor also lead to their decrease. In acute ischemia, those inflammatory factors activate fibroblasts, which enhances the ability to synthesize and secrete collagen, while some fibroblasts also undergo degeneration and necrosis, causing a release of cellular detritus, which further induces the production of a large amount of collagen fiber and their deposition. Such myocardial tissue remodeling only ceases after the tissue environment reaches a balance between the inflammatory factors and fibroblasts. Inhibition of this dynamic process and acceleration of the time-to-balance thus causes less myocardium to lose its original s and thus functional loss.

We found that IL-6R inhibitor injection lead to a significant reduction of the observed expression of IL-6 and TGFβ1 (LIILR group). Thus, the detrimental effect of TGF-beta1was reduced, i.e., the collagen content was reduced in the damaged infarction area, avoiding further sequelae. The fibrotic effect of TGF-beta 1 is much more evident in another disease, strictly linked to myocardial infarction that is NAFLD, as evident in previous studies. Also in the aforementioned disease (NAFLD), the pro-inflammatory cytokine, i.e., IL-6 plays a key role, in the sense that is increased its serum concentration, likely inducing the production of a large amount of collagen fiber and their deposition via TGF-beta. In the presence of IL-6, the role TGF-beta1 in subverting Th1 and Th2 differentiation for the generation of IL-17-producing T cells.

TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Il-17 is also central to the atherosclerosis process, driver of myocardial infarction.

Matrix metalloproteinase (MMP) belongs to the Zn-dependent protease family and can degrade extracellular matrix (ECM) components. The tissue inhibitor of metalloproteinase (TIMP) acts in the opposite way to MMP, which inhibits the degradation and thus regulates the content of ECM. The factors that influence the dynamic balance of MMP and TIMP are generally considered to be mainly TNF-α, EGF, TGF-α and the like. Upregulation of the expression levels of these factors leads to an increase in the ratio of MMP/TIMP, ie, the rate of degradation is greater than the rate of synthesis, resulting in a decrease in ECM, resulting in interstitial filling of the fibers, interstitial fibrosis, and eventually ventricular remodeling; Indeed , we observed that after treatment with IL-6, the level of MMP2/9 in the homogenate of myocardial tissue was also increased, and the IL-6 receptor inhibitor treatment significantly reduced the two indexes, local tissue fiber content. There are similar changes. This indicates that, unlike the common tissue necrosis factors such as TNF-α, EGF, and TGF-α, the inflammatory mediator IL-6 is also an important factor affecting ECM in infarcted hearts. ECM synthesis and degradation of imbalance are important factors leading to organ fibrosis. This further suggests that IL-6 may also affect infarct remodeling by mediating MMP2/9 expression.

Conclusion

The results of this study provide a new therapeutic target for left ventricular remodeling after clinical myocardial infarction.

Chapter 20. XPro1595 in Myocardial Dysfunction

"XPro1595 Decreases Myocardial Dysfunction After Resuscitation in a Rat Model of Cardiac Arrest." *In press in Cell press, Heliyon 2023, Biscof et al.*

Background/Aims: Tumor Necrosis Factor-α (TNF-α) modulates the myocardial dysfunction (MD) that occurs after cardiopulmonary resuscitation (CPR) following a cardiac arrest (CA) event. XPro1595 is a natural compound that competitively interacts with the soluble form of TNF-α to prevent it from stimulating the TNF-α receptor 1 (TNFR1), thus, reducing myocardial dysfunction and preventing further cardiac damage. However, whether this partial protection also exists in patients receiving CPR post CA needs further investigation. Here, our goal was to investigate the XPro1595-mediated potential protection of cardiac injury after CA and CPR.

Methods: CA was induced in adult Sprague Dawley rats (8 min ventricular fibrillation (VF), then 8 min CPR), before exposure to XPro1595 (10 mg/kg, I.V.) or vehicle 10 min later. The same animals received sham surgery, without VF and CPR. A computer-based data acquisition system was employed for the constant recording over four hours of the aortic and right atrial pressures, electrocardiogram, and $ETCO_2$. The ejection fraction (EF), cardiac output (CO), cardiac index (CI), and myocardial performance index (MPI) were evaluated using echocardiography at baseline, and at 2 and 4 hrs post return of spontaneous circulation (ROSC).

Results: All animals were successfully resuscitated. In the XPro1595 and control rats, the VF-inducing current, CPP, and number of required defibrillations showed no statistical difference. In addition, following resuscitation, the heart rates remained similar in all three groups. Mean arterial pressure was drastically reduced in all CPR groups, relative to baseline and Sham rats. However, there was no difference between the XPro1595 and control rats. $ETCO_2$ markedly decreased in control rats, relative to baseline and Sham rats, but increased to Sham group level in the XPro1595 rats. XPro1595 exposure markedly enhanced cardiac function post-CA and CPR. In addition, EF, CO, and CI at the post-resuscitation time points were drastically elevated and MPI was reduced after XPro1595 treatment, relative to vehicle administration. However, all parameters measured did not reach sham-operated levels.

Conclusion: XPro1595 can competitively bind and inhibit soluble TNF-α activity, thereby enhancing cardiac function after CA and CPR.

Introduction

Sudden cardiac arrest (CA) is a large contributor to global patient mortality and, consequently, a major public health concern. Despite an initial resuscitation success rate of 50%, the overall survival (OS) is <10% . This is likely due to the massive amount of myocardial dysfunction (MD) seen in 45-60% of patients who undergo successful CPR. Hence, MD is a principal factor in determining early death after CPR. Given this evidence, it is crucial to investigate approaches that can dramatically reduce MD post-CA and CPR in order to enhance patient prognosis and improve OS.

Events like CA and the subsequent return of spontaneous circulation (ROSC) can activate systemic inflammatory responses and whole-body ischemia/reperfusion (IR)-driven damage, which, in turn, can exacerbate MD, even after successful CPR. Tumor Necrosis Factor-α (TNF-α) is activated early during inflammation and directly leads to myocardial dysfunction following CA. Interestingly, TNF-α can be both beneficial and detrimental to cardiac health. Its action on the heart is highly dependent on two discrete receptors: TNF-α receptor TNFR1 (p55) and TNFR2 (p75). TNFR1 activation leads to cardiac destruction, particularly, in terms of hypertrophy, dilation, and diminished function, the combination of which can drastically worsen OS. Alternately, TNFR2 is associated with enhanced heart function and prolonged OS. Historically, TNF-α inhibitors like Etanercept and Infliximab inhibited both TNFR1 and TNFR2 activation. This resulted in a rapid decline in cardiac activity, followed by cardiac failure. In contrast, selective inhibition of the harmful TNFR1 can potentially enhance cardiac function and produce better OS after myocardial infarction (MI). However, the significance of TNFR1 in cardiac function after CA and CPR needs further investigation.

XPro1595 is a newly discovered natural compound that can selectively prevent soluble TNF-α from activating TNFR1. XPro1595 doesn't interfere with the transmembranal TNF-α which preferentially activates TNFR2. At present, XPro1595 is primarily used to manage neuronal diseases like Huntington's, Alzheimer's, spinal cord injury, focal cerebral ischemia or autoimmune encephalomyelitis. In addition, there are a few studies on the effect of XPro1595 on inflammatory diseases. Here, we hypothesized that XPro1595 would restore cardiac function after CPR in an established CA rat model.

Materials and methods

Our animal work received approval from the Institutional Animal Care and Use Committee of the Weil Institute of Critical Care Medicine and followed the strict animal care guidelines developed by both the National Society for Medical Research and the National Institutes of Health. For our experiments, we employed healthy male Sprague-Dawley rats (Harlan Sprague-Dawley Inc, Livermore, CA), aged 6 months, with the weight between 450-540g.

2.1. Experimental procedures

Fifteen rats were arbitrarily placed into 1 of 3 categories: (1) XPro1595 (n=5), where the rats underwent VF, followed by CPR and XPro1595; (2) Control (C, n=5), where the rats underwent VF, CPR, and saline; and lastly, (3) Sham (n=5), where healthy rats underwent sham surgery and received no VF induction or CPR. Fifteen minutes before VF induction, baseline breathing frequency and inspired O_2 fraction (FiO_2) were set as 100 breaths/min and 0.21, respectively. Our chosen CA rat model was described previously. Post VF induction, the rats were left untreated for the first 8 min and were subsequently provided with CPR for the next 8 mins. Precordial compression (PC), at a rate of 200/min, with a compression/ventilation ratio of 2:1, and equal compression and relaxation, was initiated at 8 min after VF. The compression depth was maintained at a coronary perfusion pressure (CPP: diastolic aortic pressure minus right atrial pressure) of 22 ± 2 mmHg, with $ETCO_2$ 11 ±2 mmHg. Electrical resuscitation (ER) was applied, with a maximum of 3 counter shocks of 2-joule. Once ROSC was observed, a 30-s interval of CPR was conducted prior to the administration of up to 3 ER shocks. This system of revival was

repeated for up to 3 times. In the XPro1595 and C rats, XPro1595 (10 mg/kg) and the same volume of saline sodium succinate were respectively administered to the right atrium over a 10-sec interval starting at 1 min past PC initiation. ROSC was defined as a >60 mmHg elevation in mean arterial pressure (MPA) for <5 min. Once ROSC was achieved, the M-VENT was carried out with 100% oxygen for the first hour, 50% for the next hour, and 21% until the end of the experiment. At the end of the experiment, all animals were euthanized via an overdose of pentobarbital (150 mg/kg via the femoral artery).

2.2. Measurements

Aortic and right atrial pressures, electrocardiogram, and $ETCO_2$ were constantly monitored and documented over a period of 4 h, using a computerized data collection system (DATAQ Instruments, Akron, OH). Myocardial activities like ejection fraction (EF), cardiac output (CO), and myocardial performance index (MPI), were recorded with echocardiography (HD 11 XE, Philips Healthcare, Andover, MA), with a 12.5 MHz transducer, at baseline and at times 2 and 4 h post-ROSC [18]. MPI was calculated based on systolic and diastolic capabilities and EF was a quantified measure of myocardial contraction [14].

2.3. Statistical analyses

Data are presented as either mean±SD or median (interquartile range), based on data distribution. Statistical analyses were done with either parametric (analysis of variance [ANOVA]) or nonparametric tests (Mann-Whitney U test). Timed measurements were analyzed with repeated-measurement analysis and subsequent two-way ANOVA with Bonferroni adjustments for post hoc comparisons. Pearson analysis was employed to examine the correlation between variables. $P < 0.05$ denoted significance.

3. Results

No discernible differences were observed between the baseline hemodynamics and blood analytical data among all three groups, all $P > 0.05$. (Table 1).

Group	Sham	C	XPro1595
Body weight, g	509.4±6.3	502.6±11.9	506.6±10.4
Heart rate, bpm	362±26	387±19	396±30
MAP, mmHg	132.8±11.5	144.9±14.3	139.1±11.5
End-tidal CO2, mmHg	38.1±4.6	37.3±5.1	35.6±4.6
Ejection fraction, %	73.9±3.9	74.2±4.7	72.5±7.6
Cardiacoutput, L/min	0.13±0.02	0.15±0.04	0.15±0.03
MPI	0.64±0.08	0.56±0.07	0.58±0.08

CA, cardiac arrest; MAP, mean aortic pressure; MPI, myocardial performance index.

Table 1. Baseline characteristics of Xpro1595, C, and Sham-operated Sprague-Dawley rats after cardiac arrest and CPR (X±S)

All animals were successfully resuscitated. In the XPro1595 and C rats, the VF-inducing current,

CPP, and the number of required defibrillations showed no statistical difference. CPP after CPR was 23±2.1 mmHg in the XPro1595 rats and 22.5±2.6 mmHg in the C rats. Lastly, the required defibrillation number was 1.2±0.4 in the XPro1595 rats and 1.7±0.5 in the C rats.

Following resuscitation, heart rates remained similar in all three groups. MPA was drastically reduced in all CPR groups, relative to baseline and Sham rats. However, there was no difference between the XPro1595 and C rats (Fig1A,B). $ETCO_2$ markedly decreased in C rats, relative to baseline and Sham rats, but increased to Sham group level in the XPro1595 rats (Fig. 1C).

EF, CO, and cardiac index (CI) at each time point after resuscitation were significantly decreased in C, relative to Sham rats. In particular, the EF, CO, and CI values at 4h post-resuscitation were 72.9±8.0%, 0.12±0.02L/min, and 2.15±0.40L/min/m^2in the Sham rats and 28.1±8.3%, 0.04±0.003L/min, and 0.68±0.06L/min/m^2 in the C rats ($p<0.001$ vs. Sham and CA). Alternately, MPI was drastically elevated in the C versus Sham rats (1.2±0.3 and 0.6±0.03, $p<0.001$). XPro1595, on the other hand, markedly improved cardiac function after resuscitation, relative to C. This was evident by the marked elevation of EF, CO, and CI values at 4 h post-resuscitation (43.6±4.3%, 0.09±0.008L/min, 1.48±0.13L/min/m^2 vs.28.1±8.3%, 0.04±0.003L/min, 0.68±0.06L/min/m^2in XPro1595 vs. C, $p<0.05$ vs.), and reduction of MPI (0.92±0.07 vs. 1.2±0.3 in XPro1595 vs. C) (Fig.1D-G). Lastly, the $ETCO_2$ value was strongly correlated with the EF value (r^2=0.29, $p<0.0001$) (Fig.1 H).

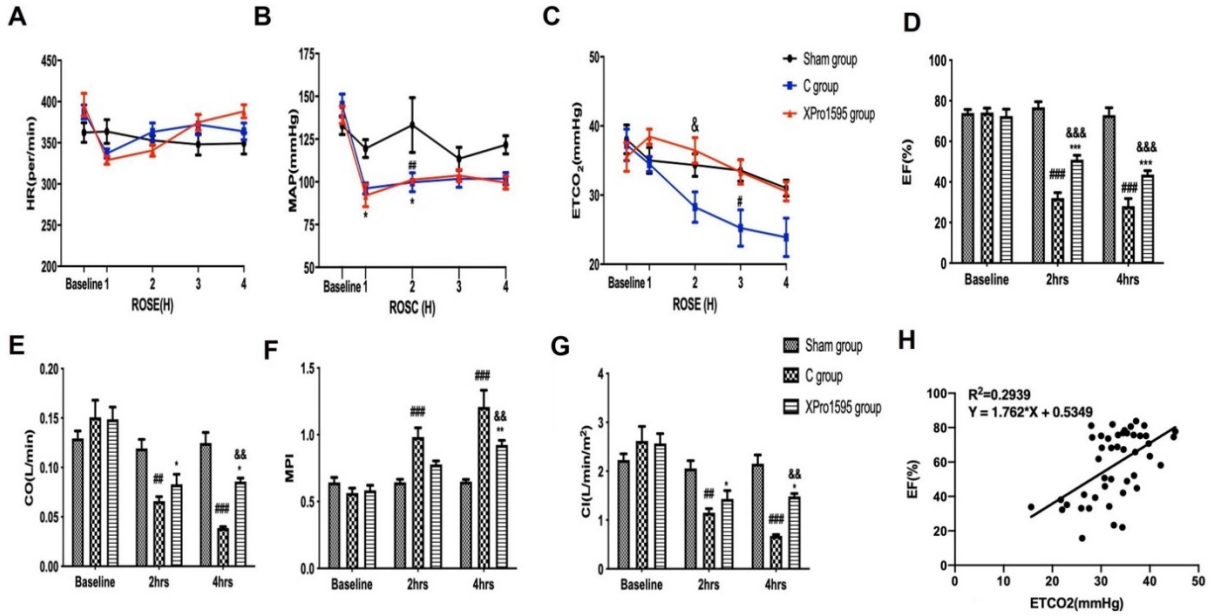


Figure 1. (A) Heart rate; (B) End-tidal CO_2; (C) Mean arterial pressure; (D) Ejection fraction, (E) Cardiac output, (F) Myocardial performance index; (G) Cardiac index; (H)Pearson analysis depicting correlation between EF and ETO_2. HR, heart rate; MAP, mean arterial pressure; $ETCO_2$, end-tidal CO_2; CO, cardiac output; EF, ejection fraction;MPI, myocardial performance index; CI, Cardiac index. Values are presented as mean±SD (n = 5 in each group). *p<0.05, **p<0.01 and ***p<0.001, XPro1595 vs. Sham group; & p<0.05, && p<0.01, &&& p<0.001, XPro1595 vs. C group; ##p<0.01, ###p<0.001, C vs. Sham group.

4.Discussion

In this study, we investigated the therapeutic outcome of XPro1595 on cardiac function in a rat model of CA after CPR. Based on our analysis, XPro1595 treatment markedly enhanced cardiac outcomes including EF, CO, CI, and MPI after CPR.

Despite major efforts to improve cardiac performance after sudden CA, the OS remains poor . The MD commonly seen after CA is the principal reason for poor survival after CA and CPR. Therefore, investigating the underlying mechanism of ROSC-induced cardiac deficit and establishing potential treatments are critical to preventing death after CPR. Hence, here we examined the effects of XPro1595, a dominant-negative inhibitor of soluble TNF-α, on post-CA myocardial function.

The EF value, for instance, declined nearly 45% at 4 h post-resuscitation, but it recovered to 60% of the baseline when pretreated with XPro1595. Furthermore, the $ETCO_2$ value strongly correlated with the EF value. Meanwhile, the CO and CI values decreased to about 30% of baseline at 4 h post-resuscitation, and increased to 75% and 69% of baseline, respectively, when pretreated with XPro1595. These results indicate that XPro1595 can restore, to some extent, the myocardial contractile performance and cardiac blood output of a post-resuscitated CA rat model. Similarly, the MPI value also increased to 2 times at 4 h post-resuscitation, but it dropped to 1.5 times of baseline after treatment with XPro1595. This further showed that both systolic and diastolic cardiac functions were prompted by XPro1595. Given this evidence, targeting the soluble TNF-α with XPro1595 may improve MD for rats after CA and CPR.

TNF-α levels rise sharply in the myocardium after ROSC and are an indicator of early mortality. In particular, its levels in plasma are strongly associated with MD after CA. Our previous animal studies also demonstrated a tight correlation between serum cytokine levels like TNF-α and MD after CA and CPR. Hence, the CPR-driven MD after ROSC induces a post-resuscitation "sepsis-like" syndrome, which is triggered by whole-body IR.

IR injury accelerates the production and release of cytokines and pro-inflammatory mediators that can simultaneously stimulate both neutrophils and coronary vascular endothelium, and promote harmful left ventricular remodeling (thinning, dilation), which can raise the possibility of heart failure.

In previous studies, TNFR1 was demonstrated to be a "death domain" that promotes cell death via apoptosis and other mechanisms. TNFR1 stimulation can also drive inflammation and cell proliferation, partially via the nuclear factor-kappa B (NF-κB) network. In a murine model of myocardial ischemia, TNFR1 suppression was shown to alleviate ventricular dysfunction and enhance OS by downregulating proinflammatory cytokines. Here, we found that XPro1595, which selectively inhibits TNFR1, partially protects against MD and improves long-term cardiac function post- CA and CPR by suppressing systemic inflammation and cardiomyocyte apoptosis.

Our work has certain limitations. Firstly, we have demonstrated the XPro1595-mediated protection against MD only in the early stage of CA.Consequently, , whether XPro1595 protects against MD in chronic heart conditions remains to be investigated . Secondly, it is still unknown whether XPro1595 could improve the survival of a post-resuscitation rat CA model.

Thirdly, additional investigations are necessary for a comprehensive understanding of the underlying mechanism(s) behind the XPro1595-mediated restoration of cardiac function.

Conclusion: The present study showed that XPro1595 can enhance cardiac function after CA and CPR by competitively binding and inhibiting the activity of detrimental soluble form of TNF-α, while saving the beneficial transmembrane form of TNF. Therefore, our data provide support for the clinical use of XPro1595 in patients with CA and CPR to improve cardiac function.

Chapter 21. Stress, diet, exercise: Common environmental factors and their impact on epigenetic age

"Stress, diet, exercise: Common environmental factors and their impact on epigenetic age" Bischof E., et al. Ageing Res Rev. 2023;88:101956. [published online ahead of print, 2023 May 19].

Aging populations are becoming a global phenomenon, with the number of people aged 65 years and older projected to reach 1.5 billion by 2050, causing collateral deficits in various socioeconomic areas, largely due to disabilities and the loss of performance in the last decades of the lifespan. These issues are best visible in developed countries, where decreasing birth rates are coupled with increasing lifespans. The list of countries with a negative demographic growth trend includes leading nations, and, as of 2022, China with its large population joined this worrisome trend. Further demographic shifts pose a serious challenge to national economies worldwide as increasing numbers of people retire and require social support and increased healthcare (Zhavoronkov, 2013).

The socioeconomic pressure has accelerated the efforts to develop and clinically implement diagnostic and therapeutic tools that would increase the healthy lifespan. The World Health Organization (WHO) has recognized the importance of addressing the challenges posed by aging populations and has launched a Global Action Plan on Healthy Aging. The plan seeks to promote healthy aging, increase access to healthcare services for older individuals, and support the development of age-friendly environments (World Health Organization, 2021).

Advancements in molecular and robotic technologies have enabled scientists to study the molecular causes of aging and form hypotheses on decelerating and even reversing this process. Aging clocks are the digital models used to quantify the aging processes and express their intensity as biological age, pace of aging, rate of aging, etc. Such models are used in (i) population studies exploring the risk factors of aging, (ii) experiments that measure the geroprotective effects of various interventions, and (iii) laboratory models in which the conventional definition of age is not applicable (e.g., in cell cultures).

The inconvenient and impractical alternative to aging clocks is observing subjects for decades until their aging pace can be derived from mortality data. In terms of testing geroprotectors as well as other interventions in longevity medicine, biological age calculation by aging clocks is the only feasible and validated solution. This type of technology is, therefore, a key piece in the search for a scientific solution to the global aging problem (Zhavoronkov et al., 2019b).

Types of aging clocks

The first aging clock using data from modern high-throughput technology was released in 2011 (Bocklandt et al., 2011). It was trained as a linear regression and validated on a collection of 128 DNA methylation (DNAm) profiles from an Illumina array, and it was able to measure a subject's chronological age with a mean absolute error (MAE) of 5.2 years. Shortly after this publication, more aging clocks followed which were trained in a similar fashion (Hannum et al., 2013, Koch and Wagner, 2011).

In 2013, the same approach was Steve Horvath applied this methodology to create a multi-tissue DNAm clock (Horvath, 2013). Horvath's clock is still being used today and has inspired many other scientists to develop their own tools to quantify aging. At the time, it featured the largest data set compiled from 82 studies and highlighted that tissues could age at different paces. The small number of 353 CpG sites used by the clock to produce accurate prediction across multiple tissues indicated that epigenetic regulation might be playing a key role in organismal aging.

Over the last 10 years, dozens of aging clocks have been developed (Galkin et al., 2020). Almost every type of biophysiological data that changes with age has been utilized to build a predictor of chronological age with the help of machine learning (Zhavoronkov et al., 2021). Currently, there are aging clocks that use clinical blood tests, facial photos, and omics data from both humans and other organisms (Bobrov et al., 2018; Fedor Galkin et al., 2020; Fleischer et al., 2018; Mamoshina et al., 2018; Meer et al., 2018; Putin et al., 2016; Zhavoronkov et al., 2020). Nonetheless, epigenetic models of aging remain popular among researchers and regularly see new developments.

While the first epigenetic aging clocks were trained with chronological age as the target variable, more recent models use alternative measures of age. PhenoAge, a 2018 epigenetic clock, used "phenotypic age" defined as a function of chronological age and a number of clinical biomarkers (Levine et al., 2018). A 2019 epigenetic clock GrimAge uses time-to-death as its target variable (Lu et al., 2019). A 2002 clock, DunedinPoAm, and its updated 2022 version, DunedinPACE, used the "pace of aging" derived from longitudinal data on the shifts in 19 clinical biomarkers as the model's target (Belsky et al., 2022, Belsky et al., 2020, Elliott et al., 2021). The models with such more elaborate definitions of the target are commonly called "second-generation" aging clocks to separate them from the models trained to predict chronological age obtained from cross-sectional studies. Nonetheless, first-generation clocks have not been replaced by these newer models and remain a popular choice among researchers. Moreover, second-generation clocks lack any shared core methodology and are based on DNAm array data, just as the first-generation clocks, which makes aging clock generations an arbitrary partition rather than a reflection of iterative improvement.

Aging clocks are a core technology in the development of geroprotective and senolytic therapies. However, despite the increasing levels of research and associated funding, longevity pharmacology has seen only limited progress (Zhavoronkov, 2020, Zhavoronkov and Bhullar, 2015, p. 11). Since 1990, governments worldwide have spent over $1 trillion on biomedical aging research (Zhavoronkov and Cantor, 2011). To secure further support for projects toward effective treatment in longevity medicine, robust criteria for the measurement of primary and further study objectives are necessary. Currently, the effectiveness of longevity therapeutics is evaluated by (i) longitudinal clinical trials focusing on mortality and (ii) trials using aging clocks (Zhavoronkov et al., 2019a). However, the latter allows much shorter iterations and, consequently, less expensive trials in the field of longevity pharmacology (de Magalhães, 2021). Some aging clocks have been patented which makes them ready for integration into existing commercial systems and further validation on a wide scale (Aliper et al., 2020, Galkin et al., 2022c, Horvath, 2021).

Epigenetic aging

Aging is a systemic process that impacts all levels of biological systems: organs, tissues, cells, and their molecular components. The hallmarks of aging, now numbered 12, capture the current main domains of aging-related mechanisms associated with aging as a comprehensive entity (López-Otín et al., 2023, López-Otín et al., 2013). The phenotypic markers of aging are easy to observe in the elderly, especially those with frailty and obvious somatic and neuropsychological deficits. However, defining an elderly or a youthful epigenetic profile is challenging.

The epigenetic landscape determines gene expression intensity and is formed by an intracellular apparatus of chromatin remodelers and DNA modifiers such as DNA methyltransferases (DNMTs). Although DNMTs are not the only epigenetic factors, their impact on overall cellular phenotype cannot be underestimated (Fig. 1). For example, hypermethylation of the promoters of tumor suppressor genes has long been recognized as a driving force in multiple malignancies(Cheng et al., 2019; Herman et al., 1994; Yazici et al., 2020). Somatic mutations in blood cells' DNMT3A have been associated with a 3.0–3.8 increase in epigenetic age further validating the importance of epigenetic regulation in aging (Robertson et al., 2019).

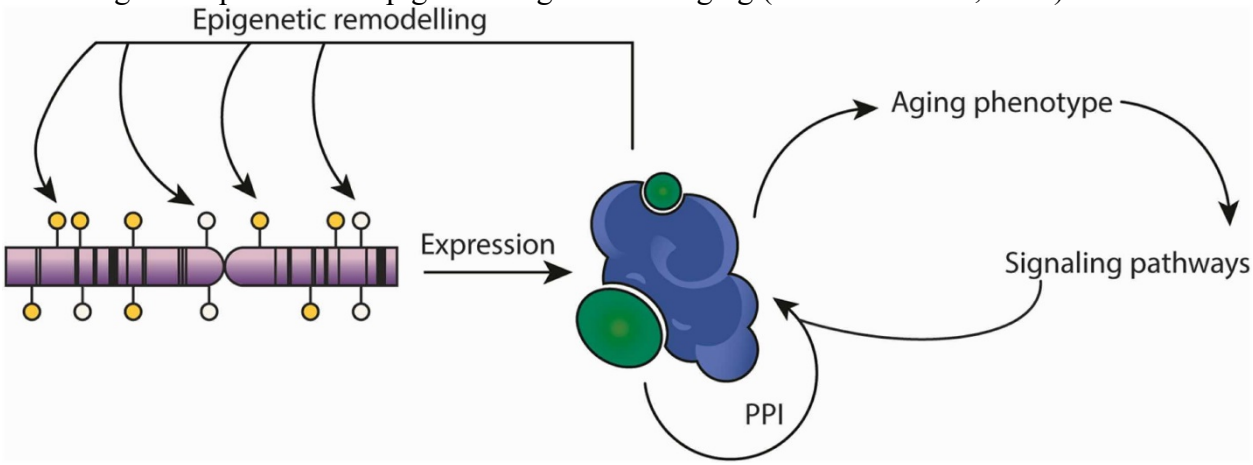


Figure 1. The aging phenotype is effectuated by proteins that either fail to perform their original function or gain new adverse functions. The manifestations of the aging phenotype include systemic inflammation, low resilience to oxidative stress, and the accumulation of intracellular and extracellular waste, accompanied by a major disruption of signaling and protein–protein interactions (PPI). Epigenetic phenomena affect the proteome by regulating gene expression, but epigenetic profiles are also affected by proteins such as DNMTs. The full understanding of the aging process cannot be complete without the full knowledge of the role of the epigenome component in it. Aging clocks are perfect tools that allow us to study the links between the epigenome and the rate of aging.

Much research is being done on the role of epigenetic mechanisms in aging, particularly the role of DNAm. It is hypothesized that the environmental stress accumulated over a lifetime disrupts epigenetic profiles and causes noisy transcription, which contributes to the aging phenotype (Zhang et al., 2020). Although the loss of epigenetic signatures is a major contributor to aging, recent findings have confirmed that these changes can be modified in animal models to reverse aging (Yang et al., 2023).

Influence of the environmental factors was best determined so far in twin studies, some of which reported 70–80% of epigenetic variance to be attributed to external influences (Cheung et al., 2018; van Dongen et al., 2016). Interestingly, epigenetic variance is more pronounced in older twin pairs, which is in line with the hypothesis of stress accumulation (van Dongen et al., 2016).

Single-cell observations also show that intra-tissue epigenetic heterogeneity is a consistent indicator of aging, further supporting this hypothesis (Cheung et al., 2018).

One study has illustrated the stochastic nature of age-related epigenetic changes as an increase in the Shannon entropy of methylation levels (Hannum et al., 2013). In other words, DNAm levels at most CpG sites tend to lose their initial hypo- or hyper-methylated identity and gravitate toward moderation (50%) with age.

Age-related epigenetic changes contribute to the aging phenotype by promoting genomic instability, carcinogenesis, and cardiovascular pathologies (Pagiatakis et al., 2021). While human studies on this topic are limited to epigenome-wide association studies, experiments on model organisms find much more conclusive evidence for the driving role of DNAm in aging (Gonzalo, 2010, Saul and Kosinsky, 2021). Recent research has focused on identifying specific epigenetic changes associated with aging as well as the molecular mechanisms that drive these changes. Besides the DNAm landscape alterations in aging individuals, histone modifications and noncoding RNA expression were reported to change as well.

In mice, negative aging-related traits can be transferred to offspring via epigenetic inheritance. More specifically, the offspring of older mice tend to have a 6.6% shorter life expectancy and develop a phenotype of an aged individual (heart fibrosis and muscle atrophy) earlier than the offspring of younger mice(Xie et al., 2018). The offspring of older murine fathers also have impaired learning and memory abilities. A similar observation has been made for humans: advanced paternal age results in an increased risk of autism, schizophrenia, and dyslexia(Saha et al., 2009). These phenotypes have been linked to hypomethylation and the consequent activation of mobile genomic elements in older sperm. Other effects of epigenetic aging include abnormal methylation patterns across the mTOR-signaling and immunological pathways.

The importance of epigenetics in aging is further emphasized by epigenetic rejuvenation studies, which have shown that modifying DNAm patterns can lead to tissue regeneration and prevent cellular senescence(Lu et al., 2020).

Thus, certain epigenetic patterns created by environmental factors can accelerate aging. Most commonly, such factors are studied in cross-sectional settings. Longitudinal observations and twin studies may also provide more reliable validation of the epigenetic aging contributors, which can ultimately lead to clinical trials aiming to provoke organismal rejuvenation or treat aging-associated conditions, such as cancer (Nepali and Liou, 2021, Wang et al., 2022).

Epigenetic therapeutics

The interest is increasing toward the potential use of epigenetic therapies to delay or reverse aging-related changes. Some studies have shown that modifying the epigenome can improve age-related declines in cellular function and delay the onset of age-related diseases, suggesting that epigenetic changes may be a promising target for antiaging interventions(Cheng et al., 2019; Meiliana et al., 2022; Zhang et al., 2020).

Epigenetic clocks can be used to quantify the influence of specific stressors and protective factors on the pace of aging (Fig. 2). In certain cases, it may even be possible to extrapolate

preexisting knowledge about the epigenetic effects of a compound or an activity to estimate its influence on aging mechanisms. In this way, epigenetic studies enable the discovery of novel therapies aimed at extending human healthspan (Zhang et al., 2020) and preventing the onset of noncommunicable diseases.

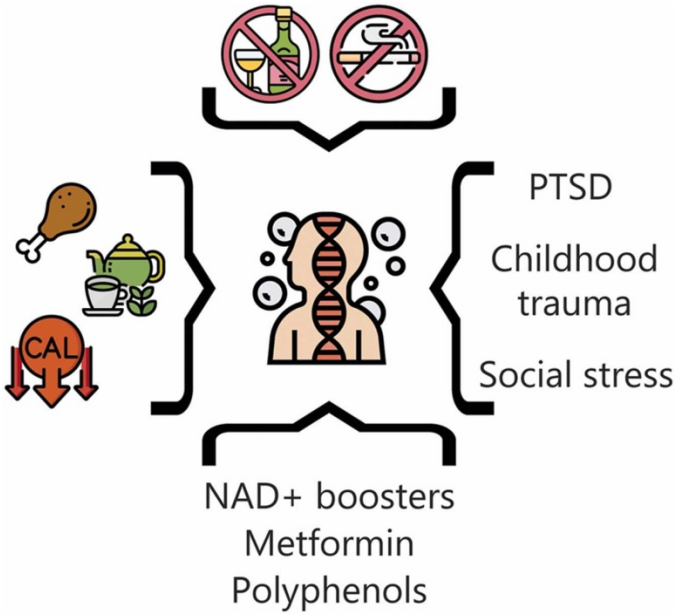


Figure 2. The epigenetic pace of aging is affected by multiple factors, including physical, chemical, psychological, and social. The nutritional studies discussed in this review show a quantifiable effect on the part of certain types of food and calorie restriction on human and animal aging epigenomes. The effects of tobacco and alcohol use on the acceleration of aging have also been studied in depth. Psychological factors and the effects of specific dietary supplements, however, are still being researched, and their contribution to epigenetic aging remains unclear.

While epigenetic clocks have also been applied to validate the effect of such interventions as epigenetic reprogramming and heterochronic parabiosis, we limit the scope of this review only to the interventions that can be enacted by longevity enthusiasts today. Epigenetic reprogramming is a promising new technology that enables the cultivation of host-derived stem cells to be used for transplantation. The de-differentiation to the pluripotent state has been associated with an erasure of epigenetic age, making the cells indistinguishable from embryonic stem cells. Some solutions involve epigenetic rejuvenation without full de-differentiation, which is considered safer due to lower malignancy risks (de Lima Camillo and Quinlan, 2021, Simpson et al., 2021, Singh and Newman, 2018). Epigenetic reprogramming and rejuvenation are promising technologies currently tested in model organisms but their application in humans is not yet feasible. Similarly, heterochronic parabiosis is studied only in model organisms due to technical and legal difficulties (Pamplona et al., 2023, Zhang et al., 2021). Even less invasive procedures such as umbilical cord plasma transfusions, despite the possible anti-aging effects (−0.82 years according to GrimAge), can be encountered only in research settings (Clement et al., 2022). For now, consumers are limited to dietary and lifestyle anti-aging therapies, whose effects will be discussed in the sections below.

Negative influence on the epigenetic age

Within the framework of longevity medicine, the benefit of interventions can be quantified with a decrease in the aging rate as measured by various aging clocks. For example, epigenetic and other clocks (especially hematologic clocks) allow clinicians to determine exactly how much younger a smoker's phenotype would be if they had quit smoking at a certain point in the past. The DNAm changes associated with smoking have been linked to previously recorded smoking-associated alterations in gene expression that include the activation of senescence genes(Li et al., 2015; Walters et al., 2014). Smoking also affects DNAm levels in other tissues such as blood(Tsaprouni et al., 2014).

Giving up smoking is one of the most common medical recommendations for all age groups(Stead et al., 2013). Several studies have shown the adverse effects of smoking to be partly mediated by epigenetic mechanisms. More specifically, smoking increases the epigenetic age of airway epithelial and lung tissues by four to five years(Wu et al., 2019). Smoking cessation at an early enough stage has the potential to enable the restoration of the normal epithelial aging rate in the airways, yet the lungs of ex-smokers maintain the footprints of aging acceleration.

Some studies indicate that smoking in youth results in irreversible damage that can be detected in later life (Klopack et al., 2022). A time-to-death clock, GrimAge, is accelerated by both smoking in youth, pack-years, and parents' smoking. In the same study, two other clocks (DunedinePoAm, PhenoAge), however, were only affected by adult pack-years. Knowledge from such biogerontological studies can be used in clinical practice to estimate whether smoking cessation could restore a patient's normal pace of aging. Another common health recommendation is to reduce alcohol consumption due to its detrimental effects on the cardiovascular and neural systems (Mende, 2019). Several recent studies examined epigenetic age acceleration in regular drinkers (Bøstrand et al., 2022). One of these finds that people with alcohol use disorder are on average 2.2 years biologically older than healthy controls(Luo et al., 2020, p. 2). The type of alcohol consumed appears to play a significant role in alcohol-induced epigenetic aging. While liquor, beer, and total alcohol amount are associated with higher biological age, wine consumption displays no such association (Nannini et al., 2023). The authors suppose that this observation might be linked to the high polyphenol content in wine, while liquor contains alcohol in high concentrations and is depleted in polyphenols.

Interestingly, the severity of aging acceleration has been associated with polymorphism in the gene APOL2 and, possibly, its expression level in the hippocampus. APOL2 also has known links to addiction and schizophrenia, which implies that accelerated epigenetic aging might also be a symptom of some mental and substance abuse disorders (Lehrmann et al., 2006).

It should be noted, however, that the measured effects vary among aging clocks and some may return statistically insignificant results (Kresovich et al., 2021b).

Epigenetic diet

Giving up smoking and alcohol are effective in decreasing accelerated biological aging. However, those lifestyle interventions are by far insufficient for longevity medicine practice.

Studies on the dietary factors affecting epigenetic aging have a wider reach and can be used to discover potential geroprotectors.

The most well-known dietary intervention with a proven effect on epigenetics is caloric restriction (CR). A prolonged up-to-40% reduction in calorie intake has been shown to prevent age-related methylome changes in model organisms(Gensous et al., 2019). Even a short-term (four weeks) CR remodels the DNAm profiles of genes involved in diabetes, inflammation, and cardiovascular health-related pathways in old rats(Kim et al., 2016). Similar results have been obtained with rhesus monkeys; long-term CR delayed the onset of age-associated pathologies and greatly improved the survival rate (Colman et al., 2009). In the CR cohort, 20% of the monkeys died from age-related causes by the age of 30, while 50% died in the control cohort. After adjusting for the lifespan difference between species, a similar drop in mortality for humans would be observed at the age of 91 years(Tacutu et al., 2018).

Until recently, the efficiency of CR in humans had not been measured, although some attempts were made to extrapolate the results obtained in primate studies(Maegawa et al., 2017). A2021 human study of CR involved 43 older adult males who underwent an eight-week program combining CR with exercise, dietary supplements, and guidance(Fitzgerald et al., 2021). The regimen resulted in an epigenetic age decrease of 2–3 years, as measured by the 2013 Horvath's clock. In the 2023 CALERIE study, 128 people had their caloric intake reduced by 25% and observed for two years (Waziry et al., 2023). A slight yet statistically reliable change in the pace of aging, as measured with DunedinPACE, was observed in a dose-dependent manner in response to CR. The other two aging clocks used in the study (GrimAge, PhenoAge) were unable to detect any aging-related changes in the CR group. The 2–3% deceleration of aging detected in CALERIE was interpreted by the authors as a 10–15% reduction in mortality rate.

Some other non-CR diets have also been assessed for their ability to decelerate epigenetic aging. In an exploration of epigenetic age in 2694 adult women, their pace of aging was put in the context of adherence to four dietary indices: DASH, HEI, aHEI, aMED (Kresovich et al., 2022). These indices represent the healthiness of a diet by assigning points based on diet composition with higher scores obtained by people who consume more vegetables, fruits, protein, polyunsaturated and omega-3 fatty acids, and those who consume less alcohol, sugars, and sweetened foods (Chiuve et al., 2012, Fung et al., 2009, Fung et al., 2008, Krebs-Smith et al., 2018). While four epigenetic clocks were tested in the study, only two (PhenoAge and GrimAge) showed significant associations with diet index adherence. Interestingly, even in these two clocks the beneficial effect of healthy eating was higher in people with low levels of physical activity. For example, women with dietary scores in the upper quartile are 0.8–1.5 years younger compared to the lower quartile, based on PhenoAge estimates. In women with more than 2.5 h of weekly activity, however, this aging decelerating is not observed at all. The authors hypothesize that diet and exercise act on the same epigenetic pathways reflected in PhenoAge and thus their effects do not stack.

Although in (Kresovich et al., 2022) Horvath's (2013) clock did not detect a significant effect of diet quality on the aging rate, a more recent study has displayed its utility in a longitudinal setting. In (Fitzgerald et al., 2023), six people underwent an eight-week "methylation-supportive diet and lifestyle program" which involved breathing exercises, physical training, dietary and

supplement prescriptions, and intermittent fasting. At the end of observation, the epigenetic age of the participants was reduced by 4.60 years on average (0.0–11.0 years range). In another recent study featuring a custom clock using only 70 CpG sites from six genes, one year of epigenetic diet resulted in a 0.58 year decrease in epigenetic age (Gensous et al., 2022). Compared to cross-sectional or retrospective studies, such designs allow us to assess the efficiency of anti-aging diets and other interventions in realistic settings and measure adherence.

A study of eating habits conducted on 407 subjects showed that the consumption of fish and poultry significantly decreased the pace of aging(Quach et al., 2017). Similarly, a diet rich in fruits and vegetables, as indicated by high blood carotenoids, has also been associated with aging deceleration(Quach et al., 2017). However, due to the methodology of the study, the effect of these factors cannot be translated into a specific number of years. Similar results were obtained in another recent study conducted on 219 women who followed a two-year plant-based diet plan(Fiorito et al., 2021). The participants in the intervention arm were, on average, 0.41 years younger than their chronological age at the end of observation, although this effect may have been confounded by the increased physical activity in which some were required to engage.

Some individual compounds (polyphenols in particular) have recently gained a lot of attention from the anti-aging community due to their effects on the epigenome mediated by their interactions with DNMTs, histone modifiers, and miRNAs (Abdul et al., 2017). There are also strong indications that curcumin, a major component of curry spice, may promote favorable epigenetic changes(Benameur et al., 2021; Reuter et al., 2011). Other plant polyphenols, such as quercetin and pterostilbene, have also been associated with epigenetic remodeling(Arora et al., 2020; Busch et al., 2015). However, the effect of these compounds on DNMTs has not been quantified in the context of aging. Thus, the concept of an epigenetic rejuvenation diet is still rather vaguely understood.

The positive effect of polyphenols is considered to be partially caused by their interactions with sirtuins, a group of proteins involved in DNA repair, circadian rhythms, and stress response. In particular, SIRT1 is involved in cellular senescence and prevents telomere attrition(Arora et al., 2020). The green tea polyphenol epigallocatechin-3-gallate (EGCG) has been shown to attenuate systemic inflammation and extend rat lifespan by 8–12 weeks(Niu et al., 2013). Another common polyphenol, resveratrol—shown to inhibit senescence and boost SIRT1 expression—is found in berries, grapes, and peanuts. Finally, genistein, which naturally occurs in soybeans, is known to directly inhibit DNMT1, DNMT3A, and DNMT3A, and consequently affect the global methylation pattern.

Although some reports suggest that other foods, such as garlic, Brazil nuts, parsley, and coffee, can affect epigenetic aging, the epigenetics of nutrition at large remains an underexplored field(Lea et al., 2001; Xiang et al., 2008). In most cases, the rejuvenative potential of specific ingredients or diets has not been properly measured due to a large number of confounders. Hopefully, the widespread use of consumer epigenetic screening will bring more clarity regarding this matter.

Physical fitness and epigenetic aging

Physical fitness is one of the key factors of human longevity. Large-scale studies have shown that people who perform well during endurance training have a mortality rate that is 3–5 times lower than that of the least fit individuals of the same age(Blair et al., 1989).

The self-evident benefits of physical training may also be interpreted as aging deceleration, which can be registered with aging clocks. As such, numerous studies have explored whether the epigenetic signatures of exercise resemble those associated with a slowed pace of aging. On a genomic scale, people with a lifelong history of physical activity display lower DNAm levels on gene promoters in muscle tissue(Sailani et al., 2019). Differential methylation occurs mostly in genes involved in the electron transport chain, insulin signaling, and oxidative stress resistance. In adipose tissue, however, physical activity has been reported to increase DNAm levels on gene promoters(Rönn et al., 2013). This discrepancy may be attributed to tissue differences, but it may also reflect different experimental designs. While the muscle study was cross-sectional and involved generally active or inactive subjects, the adipose study measured DNAm levels in generally inactive subjects who had undergone a six-month training program.

This example is illustrative of some of the issues encountered in specialized aging clock studies. First, the effect of exercise on the epigenome is not uniform across the body; thus, aging clocks may not be able to detect aging deceleration in all tissues. Second, the persistence of epigenetic changes in response to physical activity is greatly underexplored. The DNAm profile acquired after a six-month training regimen may dissipate within months of cessation. These research gaps have not been addressed and greatly limit our ability to predict the long-term impact of physical activity on the pace of aging.

The effects of exercise and weight-management via diet are well-documented to reduce various measures of aging in longitudinal settings (Ho et al., 2022). For epigenetic clocks, however, few longitudinal observations are available. Although multiple studies highlight the potentially beneficial DNAm changes caused by exercise, epigenetic aging clocks commonly fail to register them as aging deceleration(Marioni et al., 2015; Sillanpää et al., 2019). In studies that detect a significant effect of exercise on aging, its magnitude greatly depends on the definition of epigenetic age(McCrory et al., 2020; Quach et al., 2017).

While exercise itself is arguably linked to the pace of aging, epigenetic aging shows a significant and reproducible association with another metric of physical fitness—body mass index (BMI). According to twin studies and studies in obese people, 10 units of BMI correspond to 1–3 years of epigenetic age across blood, liver, and adipose tissues(de Toro-Martín et al., 2019; Horvath et al., 2014; Lundgren et al., 2021). Interestingly, bariatric surgery is known to reduce epigenetic aging proportional to the drop in BMI (Fraszczyk et al., 2020). While BMI and physical activity both affect epigenetic age, the associations between adiposity and epigenetic aging are not affected by physical activity in most cases (Kresovich et al., 2021a). Thus, BMI and physical activity cannot be used interchangeably as indicators of fitness in the context of epigenetic aging.

Psychological stress and epigenetic aging

Epigenetic aging is not determined only by physical factors, such as dietary compounds or exercise intensity. Current and historical psychological states also significantly contribute to one's pace of aging.

Psychological stress has long been known to affect human longevity on a molecular level through the promotion of oxidative processes and telomere attrition(Epel et al., 2004). More recently, accumulated lifetime stress has also been shown to be imprinted on the epigenome(Zannas et al., 2015). Aberrant glucocorticoid signaling in people with high cumulative stress is hypothesized to alter the DNAm profiles of glucocorticoid response elements that coincide with the location of epigenetic aging signatures. As a result, lifetime stress may be responsible for up to 3.6 years of biological age difference between individuals. Although this study does not report any significant aging acceleration in association with childhood stress, other studies suggest that stress accumulated at any stage of life can have long-lasting effects on the epigenome. For example, traumatic stress experienced in adulthood has been associated with 2.0 additional years of epigenetic age among Dutch soldiers deployed in Afghanistan(Boks et al., 2015). Similarly, psychological stress during childhood is reported to influence the aging rate in later life(Brody et al., 2016). More specifically, children whose primary caregivers frequently display depressive symptoms are 1.8 years epigenetically older when they reach adulthood than children of non-depressed parents. In addition, directly being the victim of violence is also associated with higher epigenetic age in children(Jovanovic et al., 2017). Additionally, research has shown that interventions aimed at reducing stress such as mindfulness-based stress reduction can have a positive impact on epigenetic aging markers. For example, meditation has been shown to decrease the epigenetic age in a cumulative manner, with each year of practicing meditation being equivalent to a 0.24 year decrease in biological age(Chaix et al., 2017).

Studies in baboons hint that less chronic stress, as opposed to trauma, may also lead to accelerated aging(Anderson et al., 2021). Alpha-male baboons are, on average, one year older epigenetically than their chronological age, and upward movement along the male dominance hierarchy results in aging acceleration. Interestingly, dominance rank was not significantly associated with epigenetic age in female baboons. The authors suggest that high competitiveness at the top of the hierarchy and the associated changes in glucocorticoid levels may be the drivers of aging acceleration in alpha-male baboons(Anderson et al., 2021; Gesquiere et al., 2011).

According to studies featuring hematological and psychological aging clocks, low mental well-being may have an even more harmful effect on one's pace of aging than smoking(Galkin et al., 2022a, Galkin et al., 2022b). The insights gained from applying aging clocks to the field of psychology highlight the importance of maintaining mental health to achieve healthy longevity.

Supplements and epigenetics

Most recent surveys show that people are generally skeptical about experimenting with extreme rejuvenation technologies(Barnett and Helphrey, 2021). In a cohort of 911 adult US citizens, only one-third would take a hypothetical pill that "enabled them to live forever at their current age."

However, the desire to live a longer life in good health is more popular. Even if only every third person took a hypothetical longevity pill, longevity pharmaceuticals would remain a multi-trillion dollar market(Scott et al., 2021). For example, metformin is a potential geroprotector that has been calculated to extend the lifespan by 3–4 years when the course is started at 75 years of age(Scott et al., 2021; Wang et al., 2017). In turn, the monetary equivalent of this gain in lifespan is at least $700,000 based on the willingness-to-pay estimations.

Potential antiaging compounds such as metformin are thus an exciting opportunity for pharmaceutical companies. However, the emerging and established longevity supplements most commonly lack the evidence base for their geroprotective efficacy. In some cases, existing studies present conflicting and inconclusive statements about emerging geroprotective supplements. For example, in a recent preprint, metformin has been reported to decrease the epigenetic age of diabetic patients by 2.77 years(Man Li et al., 2021). However, an earlier study conducted in a cohort of healthy individuals did not show any signs of age deceleration due to metformin usage(Quach et al., 2017).

Other popular supplements, such as NAD+ boosters (NMN, NR, niacin, and nicotinamide) and polyphenol formulations, are also greatly underexamined in clinical trials on their effects on biological age, despite their apparent involvement in aging and epigenetic mechanisms(Nadeeshani et al., 2021; Soma and Lalam, 2022). Official clinical trials featuring aging clocks can be applied to monitor the changes in patients' aging rate and thus boost consumer confidence and the rate of adoption of antiaging therapies. In addition, the integration of aging clocks into the pharmaceutical industry practices may lead to the discovery of new classes of geroprotectors.

Challenges of epigenetic rejuvenation

While epigenetic aging clocks are a popular instrument of scientific research, they have yet to attain ubiquitary levels of use in healthcare settings. The main obstacle to their widespread use remains the prohibitively high cost of analysis. Most epigenetic clocks were trained on datasets obtained with Illumina Human Methylation 27 K and 450 K arrays(Galkin et al., 2020, p.). These platforms measure DNAm levels on approximately 27,000 and 450,000 CpG sites, respectively. A newer EPIC methylation platform was released in 2015, which has superior coverage of 850,000 CpG sites.

Over time, the high-end EPIC array has become the industry standard, and the older arrays have been discontinued. Although this scale of analysis is desirable in research settings, consumer applications require just a tiny fraction of all available CpGs of any array. For example, Horvath's clock uses just 353 CpGs, while the available Illumina platform offers almost 2500 times more CpGs. Information from any extra probes used by a platform might be discarded with no impact on an aging clock.

Another major obstacle to the adoption of aging clocks is their variety. Each aging clock defines biological age in its own way using a distinct set of CpG sites. Consequently, the associations between biological age, health conditions, and dietary and other interventions may not align with one another(Bell et al., 2019). This uncertainty is a source of consumer and institutional skepticism toward epigenetic clocks. As is the case with some studies cited in this review, only

one of the several tested aging clocks shows a significant association with an investigated factor (Klopack et al., 2022, Kresovich et al., 2021a, McCrory et al., 2020). All clocks are different in how they are trained: the characteristics of their primary domain (tissue, health conditions, age range) and algorithm (target definition, machine learning model) can limit their applications and the scope of problems they can be used to solve. Epigenetic clocks trained on blood samples exclusively may be applicable to other tissues via tissue-specific adjustments and pan-tissue clocks, such as Horvath's, are expected to perform well in multiple tissue types (Galkin et al., 2021, Hannum et al., 2013, Horvath, 2013). Nonetheless, they might struggle with picking up the aging signal in certain tissues and it is advised to use a solution that has been developed to work with a tissue in question (Horvath et al., 2018). The exact definition of biological age used by an aging clock and defined via its target variable (chronological age, phenotypic age, time-to-death, pace of aging) also carries great influence on its ability to detect the effects of various factors on one's biological age (Simpson and Chandra, 2021). Thus, choosing an aging clock to fit a research question is an important step in the practical applications of epigenetic aging clocks.

On a more fundamental level, epigenetic clocks suffer from low interpretability. The biological significance of a certain methylation level at a subset of CpG sites is difficult to establish due to our inability to manipulate such sites in controlled settings. While the role of specific genes in aging pathways can be dissected using knockout organisms or by adjusting their activity with selective compounds, that of DNAm marks is much more difficult to define. As a result, using DNAm clocks to generate therapeutic antiaging targets is a challenging task.

Moreover, differentially methylated sites identified in epigenome-wide studies of potentially geroprotective treatments may not co-localize with the few sites captured by an aging clock. In such cases, a promising compound with proven antiaging effects may have zero impact on epigenetic age. To avoid such "false negative" results, a new kind of epigenetic clock may be necessary. In standard machine learning approaches, important features are selected blindly without relying on a priori knowledge of their biological function. Perhaps a better alternative would be to train an aging clock based on the DNAm levels of the aging-associated genes.

These fundamental issues may be relieved by the development of multi-omic clocks that unite data sources of different biological origins: epigenetics, transcriptomics, and proteomics. Such clocks could highlight the causative links connecting these branches of molecular regulation and help us reach a new level of understanding regarding the aging process.

Conclusion

Different processes are involved in the maintenance of epigenetic states. In the last 10 years, multiple models have been created based on DNA methylation data. This review summarizes the general epigenetic recommendations that are available for individuals to decrease the pace of aging (Table 1). Furthermore, some of them demonstrate anti-inflammatory, antioxidant, antiangiogenic, and anticancer properties that can potentially prolong the human lifespan.

Factor	Model	Biological tissue	Setting	Effect	Ref
Alcohol	Adults with and without alcohol use disorder (AUD)	Blood	Cross-sectional	2.22-year age acceleration in AUD, which is associated with polymorphism in APOL2.	(Luo et al., 2020)
Alcohol	Young adults	Blood	Longitudinal	Binge-drinking within 30 days of the blood draw increased the epigenetic age by 1.38 years, while each day of binge-drinking contributed 0.15 years. Total alcohol, beer, and liquor (but not wine) consumption increase the aging rate.	(Nannini et al., 2023)
Smoking	Nonsmokers, smokers, and ex-smokers	Buccal and airway epithelium, lungs	Cross-sectional	Smoking increases the age of human respiratory organs by 4–5 years.	(Wu et al., 2019)
Smoking	Adults 50 years and older	Blood	Cross-sectional	PhenoAge, GrimAge, DunedinPoAm accelerated by higher adult pack years, GrimAge accelerated by smoking in youth and parents' smoking.	(Klopack et al., 2022)
Calorie Restriction (CR)	Rhesus monkeys	Brain, muscle	Longitudinal	Long-term CR improves survival rate, with a 30% reduction in late-life mortality.	(Colman et al., 2009)
CR	Healthy adults	Blood	Longitudinal	A reduction of caloric intake by 25% for a period of two years reduces the pace of aging by 2–3%	(Waziry et al., 2023)
Healthy eating	Non-Hispanic White women	Blood	Cross-sectional	Better diet quality can reduce the epigenetic age by 0.8–1.5 years. The effect is observed with PhenoAge and GrimAge, but not clocks described in (Horvath, 2013) and (Hannum et al., 2013). Age deceleration is less significant in women who exercise more than 2.5 h per week.	(Kresovich et al., 2022)
"Methylation-supportive diet and lifestyle program"	Adults 46–65 years old	Blood	Longitudinal	Eight weeks of exercise, supplements, and dietary prescriptions reduced the epigenetic age by an average of 4.60 years	(Fitzgerald et al., 2023)
Mediterranean diet	Adults 65–79 years old	Blood	Longitudinal	After one year of an elderly-tailored Mediterranean diet, the participants reduced their epigenetic age by 0.58 years.	(Gensous et al., 2022)
General lifestyle	Adult males	Saliva	Longitudinal	The regimen resulted in a 2–3-year decrease in epigenetic age.	(Fitzgerald et al., 2021)
Fish and poultry, fruit and vegetables	Adult men and postmenopausal women	Blood	Cross-sectional	Significant beneficial effect in a multivariate linear model of biological age.	(Quach et al., 2017)
Curcumin	-	-	Review	Reduction in tumor growth via the inhibition of telomerase activity; regulation of histone deacetylases, histone acetyltransferases, DNA	(Benameur et al., 2021, Reuter et al.,

Factor	Model	Biological tissue	Setting	Effect	Ref
				methyltransferase I, and miRNAs.	2011)
Polyphenols	-	-	Review	Effects on SIRT1, DNMTs, and local methylation levels.	(Arora et al., 2020)
EGCG	Wistar rats	Serum, liver, and kidney tissues	Longitudinal	Lifespan extended by 8–12 weeks via a mechanism involving long-term consumption of this polyphenol.	(Niu et al., 2013)
Obesity	Human twins and obese people	Adipose tissue, blood, liver	Cross-sectional / Longitudinal	10 units of BMI correspond to 1–3 years of epigenetic age, according to Horvath's clock	(de Toro-Martín et al., 2019)
Obesity	Adult people	Liver, blood, adipose tissues	Cross-sectional / Longitudinal	Each 10 kg/m^2 in BMI increase epigenetic age by 3.3 years in the liver but not other tissues, according to Horvath's clock. The aging rate in the liver is not affected by bariatric surgery, the rate in adipose tissue is unaffected by exercise.	(Horvath et al., 2014)
Psychological stress	Premenopausal women	Blood	Cross-sectional	Lower telomerase activity and age acceleration.	(Epel et al., 2004)
Lifetime stress	People from an urban, African American cohort	Blood	Cross-sectional	Glucocorticoid-induced epigenetic changes and consequent aging acceleration.	(Zannas et al., 2015)
Chronic stress	Wild baboons in Kenya	Blood	Cross-sectional	The stress of a highly competitive environment increases the pace of aging in alpha-male baboons.	(Anderson et al., 2021)
Plant-based diet	Postmenopausal women	Blood	Longitudinal	A two-year diet plan has reduced epigenetic age by 0.41 years.	(Fiorito et al., 2021)
Meditation	Long-term meditators	Blood	Cross-sectional	Meditation beneficially reduced the pace of aging in all age groups. Each year of practice reduced the epigenetic age by 0.24 years in long-term meditators older than 52 years. No such effect was observed in younger subjects.	(Chaix et al., 2017)

Table 1. An overview of aging studies discussed in this review.

Although the exact magnitude of the effects that different lifestyle components exert on epigenetic aging remains undetermined in most cases, the widespread adoption of aging clocks can bridge this knowledge gap and, ultimately, enable a new mode of healthcare decision-making to fight the problem of global population aging.

Part VIII. Precision senocardiology: AI-based drug discovery and clinical trial settings

Chapter 22. New era of AI-driven drug discovery.

"The potential of rapalogs to enhance resilience against SARS-CoV-2 infection and reduce the severity of COVID-19." Bischof E, et al. Lancet Healthy Longevity 2021 Feb;2(2):e105-e111.

Introduction

The first case of infection caused by the novel coronavirus COVID-19 was reported in Wuhan City, China, in December 2019. On March 11, 2020, the World Health Organization declared COVID-19 a global pandemic. Since that time, COVID-19 has impacted the lives of billions of people; as of November 2020, it is estimated that nearly 60 million people have been infected and 1.4 million have died due to COVID-19. After a leveling off of the rate of new infections and deaths after the first wave, the infection incidence is now again rapidly growing, as are the concerns regarding the ongoing "second wave" and potential further waves, as well as long-term post-infection and post-recovery effects. Globally, we are experiencing geographic redistribution of hot spots and are left with the distinct possibility that outbreaks may reoccur not only in the months, but perhaps years ahead.

As with other viral infections such as influenza, the elderly are at a significantly increased risk of suffering adverse outcomes from COVID-19. While it remains too early to know the extent to which age impacts the risk of initial infection, it is clear is that once infected, age is by far the greatest risk factor for severe complications and death. Data from the United States Center for Disease Control (CDC) reveals that the risk of dying from COVID-19 increases roughly 10-fold for every 20 years of age. This is comparable to the relationship between age and risk of death from Alzheimer's disease.

We have postulated that the relationship between chronological age and COVID-19 mortality is driven primarily by the biological mechanisms of aging, a concept which has recently become more widely appreciated. At both the cellular and molecular levels, these mechanisms have been described as the "hallmarks"[5] or "pillars" of aging. Previous research has revealed that these hallmarks can be directly linked to the age-associated loss of immune function concomitant with increases in systemic inflammation (also referred to as inflammaging). Inflammaging can been seen in the form of aberrant activation of innate immune mechanisms, such as elevation of pro-inflammatory cytokines and increased numbers of natural killer cells, which itself may exacerbate the increased risk of viral and bacterial infections that are associated with age. Impairment of immune function is also a likely contributor to additional age-associated problems, including higher prevalence of autoimmune disorders and increased risk for numerous types of cancer due to impaired immune surveillance.

It is well established that the immune system loses its efficacy with increasing age. Immunosenescence affects both innate and acquired immunity and dramatically reduces the

production of naive T- and B-cells in the thymus and bone marrow. As a result, decreased antibody production leads to fewer T- and B-cellular interactions, as well as a limited release of thyroid hormones, thus leading to decreased natural killer cell activity and a functional decline in the body's ability to mount an immune response. The elderly are known to have a chronic low-threshold proinflammatory status along with elevated plasma markers (e.g. interleukin-6, tumor necrosis factor-α, and C-reactive protein) in the absence of clinical symptoms. On a cellular level, this translates to enhanced inflammatory activity, especially in monocytes and macrophages (i.e., the innate immune system) that work to reciprocally enforce the ongoing inflammaging processes.

The collective outcome is a compromised immune response, and a higher incidence of inflammatory comorbidities, for example, cancers and age-related neurodegeneration, which further serve to weaken the immune system. The innate immune system, which is primarily involved in the response to new infections, also suffers from a reduction in clonal diversity. This 'vicious cycle' of inflammaging and immunosenescence is believed to underlie the adaptive processes which exacerbate the severity of symptoms in aged individuals who tend to exhibit an enhanced susceptibility towards infections along with a diminished reaction to vaccines. Therefore, we and others propose that novel and effective strategies for combating COVID-19 can be developed by directly targeting the hallmarks of aging to prevent or diminish inflammaging and immunosenescence.

mTOR inhibition increases lifespan and health in preclinical models

Strong support for the premise that immunosenescence can be reversed by targeting biological aging comes from studies of the mechanistic target of rapamycin (mTOR) pathway. The mTOR protein is a nutrient- and stress-responsive kinase that functions as a conserved regulator of aging in eukaryotes. Activation of mTOR promotes development and growth, while genetic inhibition of mTOR increases lifespan in yeast, nematode worms, fruit flies, and mice. The mTOR kinase acts in two distinct protein complexes: mTOR complex I (mTORC1) and mTOR complex 2 (mTORC2). In the context of biological aging, inhibition of mTORC1 is consistently associated with increased lifespan, while inhibition of mTORC2 is associated with reduced lifespan, at least in mice. mTORC1 regulates several key homeostatic processes including autophagy, mRNA translation, and metabolism, each of which impact the hallmarks of aging and hence the lifespan of different model organisms.

The macrolide antibiotic rapamycin is an allosteric inhibitor of mTORC1 that acts by binding to the FK506 binding protein (FKBP12). Similar to genetic inhibition of mTORC1, rapamycin has been shown to increase lifespan in yeast, worms, fruit flies, and mice. The effects of rapamycin on lifespan appear to be quite robust in mice, with lifespan extension being reported in multiple strain backgrounds across a broad dose range involving both oral delivery and intraperitoneal injection. Lifespan extension is comparable when treatment is initiated at young age, in mid-life, or transiently in late life. Intermittent treatment with rapamycin in late life has also been shown to be effective at extending lifespan. Importantly, the effects of rapamycin extend beyond increasing lifespan in mice, with evidence for reduction in hallmarks of aging. This includes fewer age-related cancers, protection against cognitive decline, improved cardiovascular function,

restoration of immune function, improved renal function, oral health, enhanced intestinal function and reduced gut dysbiosis, and preserved ovarian function.

Other pharmacological inhibitors of mTOR have been described, but there is relatively little data on their effects on lifespan or health during aging. In general, existing mTOR inhibitors can be classified into three categories: rapamycin derivatives (rapalogs), other mTORC1-specific inhibitors not structurally related to rapamycin, and ATP-competitive inhibitors of mTOR. Rapalogs and other mTORC1-specific inhibitors are generally predicted to function similarly to rapamycin to enhance lifespan and improve age-related phenotypes; however, to date, only everolimus (also known as RAD001) has studied in this context. The evidence supporting geroprotective effects from everolimus include improved muscle function during aging in rats and, as discussed below, improved immune function in healthy older people. ATP-competitive inhibitors which inhibit both mTORC1 and mTORC2 usually have off-target effects on other kinases. Examples of ATP-competitive inhibitors of mTOR include Torin 1, Torin 2, and the PI3K/mTOR dual kinase inhibitors such as dactolisib (also known as BEZ235 or RTB101). To our knowledge, there is limited data supporting the positive effects of ATP-competitive mTOR inhibitors on lifespan in any animal model, and only rapamycin has been demonstrated to increase lifespan in mice.

Inhibition of mTOR reverses age-related decline in immune function

Although rapamycin and rapalogs have traditionally been considered as immunosuppressives, multiple studies have indicated that rapalog monotherapy is sufficient to reverse age-related declines in immune function in both mice and people. One of the first studies to demonstrate the effectiveness of rapalogs was in a mouse model of age-related immune senescence. In that study, 22-24-month-old mice were treated with either rapamycin or a vehicle control for a period of 6 weeks. After a two-week washout period, mice in each group were immunized against the H1N1 strain of influenza. Two weeks later, each group was challenged with live H1N1 and their survival was quantified. When compared to young mice, the aged mice that did not receive rapamycin showed a dramatic reduction in their ability to respond to the vaccine, with approximately two-thirds of the animals failing to mount an immune response and dying within 10 days of H1N1 challenge. In contrast, aged mice that received rapamycin exhibited improved immune function, with all of the rapamycin-treated animals responding to the vaccine and surviving the subsequent H1N1 challenge past the endpoint of the experiment. This functional rejuvenation was correlated with a decrease in senescence markers in hematopoietic stem cells along with improved stem cell function[49], although the precise mechanism of action remains to be established.

This preclinical work spurred efforts to assess whether similar outcomes would be seen in a clinical setting. Two Phase 2 clinical trials have been completed where older adults of normal health were treated with everolimus alone or everolimus combined with RTB101 for six weeks. Both studies were randomized, placebo-controlled and found that patients who were given the rapalog demonstrated improved responses to influenza vaccine compared to those who received placebo-only. Intriguingly, in the second study, patients that received everolimus + RTB101 also had fewer infections over the following year, suggesting that the immune-boosting effect may extend beyond the initial vaccine response. Enhanced autophagy because of mTOR inhibition

along with increased expression of anti-viral proteins have been proposed as potential mechanisms of action for the observed immune-boosting effects in people. A subsequent Phase 3 clinical trial using RTB101 alone failed to meet its endpoint.

The observation that immune function can be improved over a period of several weeks to months following a single 6-week interval of mTORC1 inhibition has important clinical implications. Influenza alone is estimated to result in 300,000 to 500,000 deaths annually, with elderly individuals at highest risk. Improving vaccine response among this vulnerable population could substantially enhance preventative measures and lead to less severe clinical outcomes. A transient treatment regimen is also likely to be more easily adopted across large cohorts and have substantially fewer adverse effects compared to chronic high-dose regimens taken by organ transplant patients. Indeed, no significant adverse events were noted in either of the Phase 2 mTOR inhibitor trials, and there is growing evidence that low dose rapalog monotherapy has minimal side effects in adults of normal health status. These findings are further supported by the lack of observed side effects in non-human primate marmosets[66] and in older companion dogs treated with lower doses of rapamycin thought to be consistent with delayed aging.

It is also worth noting that a restoration of immune function in older adults is likely to have benefits that extend beyond simply boosting the response to an influenza vaccine. Prior to COVID-19, respiratory infections were estimated to account for more than a million deaths in adults older than 70 years, and more than two million deaths in people of all ages annually worldwide, a number that will undoubtedly be much higher in 2020. Additionally, it is expected that enhanced immune function would lead to reduced rates of age-associated cancers, as immune surveillance is known to be a critically important anti-cancer mechanism that is impaired by the aging process. Reversion of age-related changes in the microbiome could also be expected following mTOR inhibition, as the immune system plays a critical role in maintaining a "healthy" microbiome. Intriguingly, rapamycin has been found to reduce age-related cancers and modify the aged microbiome in mice, although it remains to be established whether these effects are mediated by the immune system.

Evaluating the feasibility of rapamycin for COVID-19 prevention

The most important consideration for any clinical intervention is to evaluate the potential benefit against the potential risk. This is always challenging to quantify, but is even more difficult when considering a treatment that is preventative in nature and given to individuals who are not currently sick. As discussed above, the potential benefits to preventing immunosenescence in the elderly are quite large and include reductions in morbidity and mortality from infectious disease and cancer. In the case of COVID-19, extrapolation from animal models suggests that the immune restorative properties of rapamycin might be expected to reduce COVID-19 deaths substantially in the absence of a vaccine and possibly by an even greater amount once a vaccine is widely available.

Due to the abundant clinical data on rapamycin use (also known as sirolimus or Rapamune), we can also predict what the likely risks are. Rapamycin and other rapalogs (everolimus, temsirolimus) have been most widely employed to prevent organ transplant rejection but are also approved for use in lymphangioleiomyomatosis, coronary stenting, and certain forms of cancer.

Use of high doses of rapamycin by organ transplant patients is associated with numerous side effects including (≥30% occurrence, leading to a 5% treatment discontinuation rate) generalized pain, headache, fever, hypertension, nausea, abdominal pain, constipation, diarrhea, urinary tract infection, peripheral edema, anemia, arthralgia, thrombocytopenia, hypercholesterolemia, hypertriglyceridemia, and increased creatinine. Side effects are mostly reversible and represent a worst-case scenario, as these are sick people taking high doses of the drug along with other medications. Risk of serious complications, even from acute overdose with rapamycin, are extremely low. For this reason, we believe that short-term treatment (up to a few months) with low doses of rapamycin will have minimal adverse events and that the risk/reward ratio strongly favors the potential beneficial effects from treatment.

To our knowledge, there are currently no active or planned clinical trials of rapamycin or rapalogs as a preventative treatment for COVID-19. As of November 2020, there are 214 registered not-completed clinical trials on clinicaltrails.gov using the search term rapamycin (sirolimus); six of these are related to COVID-19 (**Table 1**). In each of the existing or planned trials, rapamycin is being tested as a treatment in hospitalized patients with confirmed COVID-19. Thus, the rationale for potential efficacy in these trials, based on the ability of rapamycin to prevent the "cytokine storm" seen in very sick COVID-19 patients or its potential direct anti-viral effects is quite different from the effects of rapamycin on biological aging. The biopharmaceutical company resTORbio Inc. has initiated a small clinical trial of RTB101 in nursing home residents, to determine whether COVID-19 severity is impacted by the drug (**Table 1**). The FDA-approved endpoint for this trial is "the percentage of subjects who develop laboratory-confirmed COVID-19 with protocol-defined progressive symptoms or are hospitalized or die through four weeks of study drug treatment". While there is supportive data from the prior Phase 2 study suggesting that everolimus + RTB101 can improve immune function in the elderly, as mentioned above, RTB101 acts by a different biochemical mechanism from rapamycin and has not yet been shown preclinically to have effects on biological aging. Thus, while we are hopeful that these ongoing clinical will prove successful, none of them address the possibility that rapamycin will rejuvenate immune function in the elderly and afford protection against COVID-19 to the most vulnerable individuals.

We advocate strongly for a large-scale clinical trial in at-risk populations to test for prevention of COVID-19 by rapamycin. The rationale for such a trial is provided by the demonstrated ability of rapamycin and rapalogs to reverse age-related declines in immune function in both preclinical models and in people. Elderly patients have significantly worse clinical outcomes following infection with COVID-19, and preventative treatment with rapamycin is predicted to reduce rates of infection and positively impact clinical outcomes by reducing the number and severity of complications in biologically aged patients. We hypothesize that rapamycin will restore immune function corresponding to approximately 20 years of biological age, thereby reducing severe outcomes and death from COVID-19 infection by approximately 4-fold to 10-fold. Furthermore, enhanced immune function following rapamycin treatment is expected to improve the response to a forthcoming COVID-19 vaccine as well as provide ongoing protection against other infections that preferentially impact the elderly.

Clearly, the details of a well-designed randomized clinical trial would need to be carefully considered. These include the dose of rapamycin given, the duration of treatment, demographic

features of the patients enrolled in the study, specific endpoints to be evaluated, the duration of follow-up, and necessary cohort sizes to achieve statistical power. We have summarized some preliminary recommendations in **Table 2.** While the simplest design would include only placebo and rapamycin treatment groups, an additional multi-arm design that is worth exploring could include additional treatment with metformin. Metformin is the most widely used anti-diabetes drug in the world and is being tested for beneficial effects on aging through the Targeting Aging with Metformin clinical trial. Although the preclinical evidence that metformin can positively impact the aged immune system is less robust than for rapamycin, there is accumulating evidence that diabetics taking metformin are at reduced risk of severe outcomes or death from COVID-19 compared to diabetics not taking metformin. Further, metformin combined with rapamycin in mice is thought to improve metabolic function and slightly further increase lifespan, relative to rapamycin alone.

One innovative feature that we suggest be incorporated into a trial is the consideration of predicted biological age as an enrolment criterion. Enrolment based on chronological age is common in clinical studies, similar to the design of the rapalog trials for influenza vaccine response. However, we propose that it may be useful to consider state-of-the-art measures of predicted biological age for geroprotective clinical trials. Such biological age predictors could include estimates of epigenetic age using commonly applied epigenetic clocks and more recently described, comprehensive "deep aging clocks", based on signatures derived from blood biochemistry, imaging, transcriptomics and other types of available data. Patients could be enrolled whose biological age exceeds their chronological age by a chosen threshold (e.g. 5 years), enriching for individuals at the highest risk for negative outcomes and death and who are predicted to receive the greatest benefit from a geroprotective intervention such as rapamycin. Although we recognize that the mechanisms and predictive power of current biological age estimators have yet to be clinically validated and may present unique challenges from a regulatory perspective, there is growing consensus that these tools can provide valuable insights into underlying physiological states that influence risk for diseases of aging and for all-cause mortality. They could, however be used as auxiliary markers until clinical validation in COVID-19. For that purpose, the data required for aging clocks should be collected, such as in proposed trial.

A final consideration may be whether, even once shown to be efficacious, widespread use of a geroprotective intervention is economically feasible or justified, given the strain that many national healthcare systems are already experiencing. It now appears likely that one or more COVID-19 vaccines will be available in early 2021, perhaps reducing the incentive to continue to develop novel preventative therapies. It must be noted, however, that influenza deaths still number in the hundreds of thousands each year even with effective vaccines, and those most susceptible to severe cases of both COVID-19 and influenza are also the least likely to mount an effective vaccine response. Thus, from the perspective of cost in terms of human lives, justification for this type of approach is obvious. It is also well established that the total economic benefit from a successful geroprotective therapy far outweighs the cost of development and implementation. Work from Olshansky. Goldman and colleagues done pre-COVID-19 has estimated that the total economic benefit from such an intervention will exceed seven trillion dollars over a few decades. The total economic value of an effective geroprotective strategy is likely to be substantially greater today.

Conclusions

The novel coronavirus COVID-19 disproportionately affects the elderly and the comorbid individual, with likelihood of severe complication and death mirroring that of other age-associated diseases. Inhibition of mTOR has been shown to delay or reverse many age-related phenotypes, including declining immune function. There is an urgent need for a precision medicine trial using a functional metric of aging, investigating individuals assessed by biological age, who can then be further stratified into groups that achieve best outcomes and are benefiting from the treatment. Rapamycin and rapamycin derivatives (rapalogs) are FDA-approved inhibitors of mTOR with broad clinical utility and well-established dosing and safety profiles. Based on existing pre-clinical and clinical evidence, a strong case can be made for immediate large-scale clinical trials to assess whether rapamycin and other mTOR inhibitors can enhance resilience towards communicable and non-communicable diseases, prevent COVID-19 infection in vulnerable populations and also to determine whether these drugs can improve outcomes in COVID-19 patients.

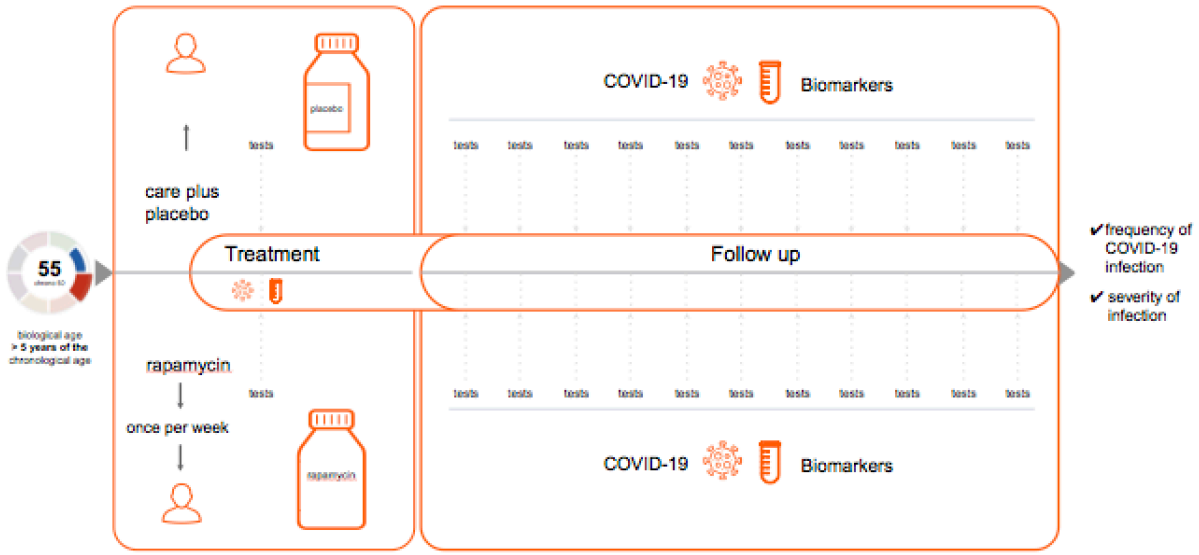


Figure 1. Representative schematic of a potential trial design. A randomized clinical trial for prevention of COVID-19 by rapamycin would likely involve transient treatment with rapamycin in older adults at high risk for COVID-19 complications over a period of several weeks. We propose that the study would selectively enroll individuals whose estimated biological age exceeds their chronological age (see main text). Following treatment, patients would be followed for several months to determine if any differences in rate of infection and severity of outcomes is observed between placebo and rapamycin treatment groups.

.

Trial name	Recruitment status	Estimated Enrolment	Study Start Date	Experimental design
Efficacy and Safety of Sirolimus in COVID-19 Infection *NCT04461340*	recruiting	40	July 25, 2020	Group A: 20 patients will receive sirolimus (oral dose of 6 mg on day 1 followed by 2 mg daily for 9 days) plus national standard of care therapy against COVID 19 Group B: Placebo + standard medical care
Sirolimus Treatment in Hospitalized Patients With COVID-19 Pneumonia (SCOPE) *NCT04341675*	Recruiting	30	April 24, 2020	Group A: Sirolimus 6mg on day 1 followed by 2mg daily for the next 13 days or until hospital discharge, whatever happens sooner. Group B: Placebo + standard medical care
Sirolimus in COVID-19 Phase 1 (SirCO-1) *NCT04371640*	Recruiting	40	May 2020	Group A: Sirolimus + standard medical care Day 1: 10mg Days 2-7: 5mg Group B: Placebo + standard medical care
Hydroxychloroquine in Combination with Azithromycin or Sirolimus for Treating COVID-19 Patients (COVID19-HOPE) *NCT04374903*	Not yet recruiting	58	May 1, 2020	Group A: Subjects will receive HCQ 600mg PO X 10 days and AZ PO 250mg DAILY X 10 days. Group B: Placebo + standard medical care
Phase 3 Study to Determine if RTB101 Reduces the Severity of COVID-19 in Older Adults Residing in Nursing Homes	Recruiting	550	July 11, 2020	Group A: 10 mg daily RTB101, mTORC1 inhibitor Group B: Placebo + standard medical care

NCT04409327				

Table 1. Clinical trials of mTOR inhibitors related to COVID-19.
A search for COVID-related clinical trials on clinicaltrials.gov was performed on October 23, 2020 with the following studies identified

General design	Double-blind, placebo controlled, randomized clinical trial
Patient population	We suggest enrolling older adults (e.g. <60 years) who are predicted to have a biological age at least 5 years greater than their chronological age (see main text).
Cohort sizes	Would be determined based on predicted infection rate and progression to severe outcomes. Likely several thousand per arm are needed.
Dose	5-10 mg rapamycin provided once per week
Duration	6-10 weeks treatment, 8-10 months follow-up
Exclusion criteria	Prior COVID-19, immune compromised, active infection
Endpoints	Rates of COVID-19 infection, severity of outcomes (hospitalization, death), vaccine response (if available)

Table 2. Initial recommendations for a clinical trial.

Chapter 23. New era of AI-driven drug discovery in Covid-19.

"The SARS-CoV-2 pandemic from the front seat of an artificial intelligence company The dawn of the pandemic and lessons from the past." Book: Biotechnology in the Time of COVID-19

In order to react more efficiently and quickly to future pandemics, to promote global collaboration, and to avoid diplomatic disputes (alt: this could be "to avoid finger pointing), it is important to carefully reconstruct, review, and record the timeline of the SARS-CoV-2 (further referred to as COVID-19) outbreak from the perspectives of all key stakeholders. On December 26th 2019, I arrived in Shanghai to work on a partnership deal with a pharmaceutical company in China. I used well-populated high-speed trains and subways, and shook hundreds of hands. On January 5th, 2020, the WHO published the first news of the coronavirus outbreak. On January 6th, I flew from Shanghai to San Francisco to attend multiple events surrounding the world's largest biotechnology and pharmaceutical convention, the 38th J.P. Morgan Healthcare Conference, which took place between January 13th and 16th. This conference attracted thousands of biopharmaceutical industry executives. In other words - the entire pharma world gathered in one city. Meanwhile, on January 12th, China publicly shared the genetic sequence of "the novel coronavirus", later named SARS-CoV2, and on the 14th, the WHO provided a detailed outbreak report. Yet, the debates at J.P. Morgan focused on more routine topics, such as the potential for AI in biomedical discovery and development, the importance of digital technologies, the use of AI for progressing research in multiple disease areas, and drug pricing; there was no mention of the coronavirus. It was only at the end of the event that some executives - those planning to go to China - started to wonder whether they would be safe. No one was talking seriously about repurposing their existing drugs to combat COVID-19, or engaging in drug discovery efforts.

At that point, the epidemic was only beginning, but its widespread ramifications became increasingly predictable. On January 23rd Wuhan underwent an official lockdown, just two days before the Chinese New Year, drawing broad criticism from Amnesty International and Western media as a human rights violation, which brought the outbreak into the public spotlight. Around January 24th, I proposed to Insilico Medicine board members, several investors, and employees a possible project on COVID-19 - a term introduced only on February 11th. While the board was in favor, some key biotechnology investors and advisors were skeptical. However, within another month, the isolated epidemics coalesced into a worldwide pandemic, as classified by the WHO on March 11th. Until then, most experts assumed that operations could continue as normal, just as they did during the SARS and Ebola outbreaks. Neither of these previous events had resulted in commercially-viable opportunities to develop effective treatments.

Internal Efforts at Insilico Medicine

While Insilico Medicine is focused on AI and is comprised of multiple interdisciplinary groups, we have five main divisions: software development, drug discovery, biology, chemistry, and deep learning theory. After a brief brainstorming session with the leaders of these divisions, taking into consideration abundant information from China, we decided to prioritize our projects by the phase of the disease, starting from generative chemistry (generating novel molecules for key viral proteins) and drug repurposing (novel preventive/therapeutic antifibrotic agents), over

repurposing and retraining AI-biomarkers of aging (as predictors of susceptibility to infection and prognosis), finally with overarching work on software (the release of COVIDOMIC, a COVID-19 version of our Pandomics.com system for patient stratification and in-the-field research, including microbiome analysis tools).

Generative Chemistry

On January 26th, I got an OK from key members of Insilico board and other team members, and began work on COVID-19. We quickly identified 3C-like protease as a prime target and applied our generative chemistry platform, which handles multiple scenarios with varying amounts of available input data, to generate 100 molecules targeting the SARS-CoV2 3C-like protease. We chose a homology modeling approach, using the protein sequence to model several variants of the binding pocket, generating a number of template molecules likely to bind the protein. Next, we expanded the chemical space using these templates and generated tens of thousands of molecules with different medicinal chemistry properties. Finally, we applied our deep learning medicinal chemistry filtering system to narrow the molecules down to the few most suitable options. Simultaneously, we worked on the 3C-like protease crystal structure, using the actual structure (Dr. Rao's lab, ShanghaiTech) in addition to homology modeling. Its small fragment served as a template to generate multiple molecules with a similar structure using ligand-base.

Here, we faced another challenge - all of our synthetic chemists were quarantined in Wuhan until the end of March! So instead of performing the synthesis ourselves, we published the 100 synthesized molecules via a dedicated page on our company's website (https://insilico.com/ncov-sprint/) and on the ResearchGate preprint server, where both authors of this chapter have key contributor status for the COVID-19 community. This resulted in numerous inquiries from medicinal chemists. After regaining our chemistry capabilities, we decided to synthesize and test a few molecules from these generations.

Repurposing of Antifibrotics, Senolytics, and Rapalogs

One of the core drug discovery efforts at Insilico Medicine is focused on lung and liver fibrosis with novel pan-fibrotic targets and molecules. We immediately looked at repurposing these for COVID-19 rehabilitation and expanded our pre-clinical efforts.

We also explored a non-traditional, novel and more controversial drug repurposing approach. Instead of looking for known molecules that interact with viral proteins, we looked for molecules that could combat immunosenescence, especially in the elderly and the comorbid population. As initial patient data emerged, it became obvious that the infection rates, severity and lethality are much higher in these two groups, while younger people experienced milder symptoms and faster recovery. With this background, we postulated that immunomodulators are urgently needed to rejuvenate the immune systems of the elderly, making them more resistant to infections and improving their ability to mount an immune response to possible vaccines. This AI-supported, longevity-based idea has thus far been well accepted among scientific, clinical and public circles. We hope this idea will extend beyond the COVID-19 era, steering research towards preventative and therapeutic strategies to increase response rates in the elderly toward various diseases, including infectious, autoimmune, and tumor diseases, among others.

Collaborations and governmental support

The multifaceted nature of the COVID-19 pandemic demands global, multilateral collaborations to rapidly advance science and technology. Unfortunately, most governmental funds, pharmaceutical companies and even special programs in innovation centers lacked the flexibility to refocus and support AI-powered companies specializing in drug discovery for COVID-19, in contrast to other fields. Insilico operates in 6 countries; however, substantial national support for our COVID-19 initiatives remained absent. Such lack of practical promotion might result in a scarcity of effective drugs targeting SARS-CoV2, of dedicated AI software systems for multi-omics drug discovery, and of comprehensive data for machine learning exploitation.

Big pharmaceutical companies either started repurposing their drugs for COVID-19, developing vaccines, or donating resources for disaster relief. However, their AI departments failed to produce any tangible papers or products quickly enough, neither did they engage with the key AI companies. This demonstrates the current state of AI in drug discovery and this trend is unlikely to change. It is up for the startups to demonstrate the real value of AI.

However, in academic circles the situation is dramatically different. COVID-19 resulted in massive academic collaborations without any funding. Very often these consortia or individual members received substantial computing resources from Amazon, Alibaba, Baidu, Google, Nvidia, and other cloud providers who should be recognized for these efforts. Insilico quickly became part of several such consortia.

In early 2019, Alan Aspuru-Guzik and I discussed the possibility of a viral or bacterial pandemic and the need to create a mitigative, multidisciplinary task force utilizing the latest advances in AI ("Antiinfective AI consortium"). Our initial idea to show a proof of concept in target identification and small molecule generation to inhibit bacterial growth was outpaced by an MIT group co-lead by Regina Barzilai. We then planned to demonstrate this concept in viruses. However, the pandemic prevented us from pursuing this further at the time. Aspuru-Guzik's lab consecutively initiated MyTrace.ca - contact tracing with Bluetooth and GPS via a mobile app. He and many other leaders formed working groups, gathering scientists together to work transparently both independently and collaboratively. This model is likely to continue past the pandemic.

AI drug discovery and the clinic

Insilico has consistently worked closely with physicians to engage them in dialogue. In China, these efforts have proved fruitful, as the country’s permanent strive to evolve and invent, coupled with the ongoing COVID-19 crisis, represent an unprecedented opportunity to test AI and precision medicine solutions for public health.

Clinicians continue to espouse mistrust of innovative technologies for use in drug discovery and repurposing. These resentments are mostly rooted in a lack of knowledge about rapidly evolving AI and machine learning. As medics (EB), we tend to fixate on the argument on changeable, adaptive biological systems and to a priori scrutinize tech visionaries. This view fails to

recognize that medicine has both a human side, as well as a scientific and technical side - being "the science of human nature" (R. Adler).

The pandemic disrupted the dismissive, skeptical attitude of clinicians, and facilitated an openness to acknowledging the utility of rigorous scientific AI tools. There is now a growing effort to integrate new technologies into the clinical practice, commencing with fast AI-supported identification of drug targets. Further, there is a new appreciation of AI as a driving mechanism of response to pandemics.

The purely empirical approach has proved suboptimal, with hundreds of studies thus far revealing no therapeutic benefit and with initially promising drugs leading to severe adverse reactions and lethality.

What will the future hold? Hopefully, a realization that AI and the clinic are inseparable entities, with the patient at the center of attention. Precision medicine cannot be achieved in any other way. Potentially the most significant transformation of all will be the shift towards a paradigm of inclusion of both human and artificial intelligence.

Lessons from China

The COVID-19 pandemic revealed China's capacity to rapidly act and react, create and innovate. Hopefully, the global community will appreciate the efforts of the scientists, doctors, and the government, rather than criticize or blame China.

The work of Chinese scientists and government laid the foundation and set an example for the world, bringing pivotal repurposing candidates, clinical trials, and a brilliant public health response. China's leaders from all domains were the first to face the novel coronavirus, operating with very limited information, including the possibility of asymptomatic transmission. And despite the fact that their response, including the complete lockdown of Wuhan and Hubei provinces and efficient contact tracing, was abundantly criticized by human rights advocates and Western media, these techniques proved to be effective and were later implemented in many other countries. The scientific community in China was also the first to respond. Within days of the outbreak, hundreds of COVID-19-related WeChat groups formed, some specifically dedicated to relevant scientists. Massive community resources emerged and became available. In January, the Global Health Drug Discovery Institute (GHDDI), an organization founded by Tsinghua University, the Bill & Melinda Gates Foundation, and Beijing Municipal Government, set up a GHDDI-AI Lab portal on GitHub, gathering all available knowledge, databases, and communities, facilitating cooperation and discussions.

Unfortunately, many scientists have no established contacts with key Chinese labs and are unaware of their research activities. In the post-COVID-19 era, we need closer East-West collaborations, courteous professional relations, and integration of research activities with Chinese scientists, whose data and expertise often surpass the West. Infectious diseases know no borders; so should collaborative efforts.

Conclusion and perspective

The pandemic has caused many social, economic and political disruptions. Hopefully, it will also disrupt the attitude of ignorance ("this is not my problem and we should not be spending the resources") which existed before COVID-19 and still does, at almost every level. The pandemic highlighted the many inefficiencies in drug discovery and development within the pharmaceutical industry. Both traditional and AI-powered methods failed to provide effective repurposing candidates, novel interventions, or vaccines for several months after the start of the epidemic.

As the pandemic subsides and the dramatic economic impact becomes apparent, it is important to avoid pointing fingers, which can hinder information and resource sharing. Instead, we should focus on fostering closer collaborations between Chinese and Western scientists. COVID-19 and other infectious diseases know no borders and nationalities, and only truly global efforts will lead to effective prevention, drug discovery, and development efforts, and technologies required for rapid responses. Many of these enabling technologies will also help us develop better medicines for non-infectious diseases, which result in a substantial global death toll.

To boost the economy in the near term, avoid the loss of tens of trillions of dollars, and prevent the loss of hundreds of thousands of lives in the future, policy makers and industry leaders must set ambitious goals, not unlike those set by the Apollo program, focusing on increasing the speed of drug discovery and development, and refocusing global efforts on biotechnology.

Adopting and advancing AI for drug discovery, driving its evolution by combining the machine and human intelligence in medicinal chemistry, is indispensable. Currently, the time it takes about 10 years to discover and develop a drug. The process costs around $3 billion and fails 90% of the time. And the resulting drugs do not work in all patients. Now imagine a world where a "driverless" AI-driven approach allows for a fully-automated drug discovery, shaving 3-4 years off the typical timeline, increases the probability of success by 50% and helps address only those patients, who will certainly benefit from the drug. This is the big goal that all of us should be striving for. In the context of global pandemics, this approach can be used to prepare the treatments for all key viral and bacterial mechanisms, prove safety, optimize mass production, and be prepared to optimize and deliver curative solutions in a matter of weeks after the next outbreak.

Chapter 24. AI for drug repurposing

"Artificial Intelligence for peer review and drug repurposing." Published in Nature biotechnology 2020 Oct;38(10):1127-1131.

The COVID-19 pandemic has transformed the way scientific and clinical results are shared and disseminated. According to a recent analysis, an average of 367 COVID-19 papers are being published every week, with a median time from submission to acceptance of just 6 days (compared with 84 days for non-COVID-19 content). These unprecedented peer review turnaround times—and in some cases relaxed editorial standards—are justifiable in a context where new information may accelerate the progress in general knowledge of and identifying solutions to the emergent global medico-socio-economic disaster, but they also bear the risk of releasing preliminary or flawed publications misleading research and development efforts, compromising clinical practice and misinformed policy makers. Can traditional computational analysis and machine learning help compensate for inadequate peer review in the context of a pandemic? Here, we propose a strategy whereby rigorous community and peer-review is coupled to the use of artificial intelligence to prioritize research and therapeutic alternatives described in the literature, enabling the community to focus resources on treatments that have undergone appropriate and thorough clinical testing.

When papers get it wrong

In 1998, Andrew Wakefield and his colleagues published a paper linking vaccinations and autism. Twelve years after that publication—a landmark study that turned hundreds of thousands of parents around the world against vaccinating their children because of the implied causal link between vaccinations and autism—*The Lancet* announced on Feb. 2, 2010 that "several elements" of the Wakefield paper "are incorrect, contrary to the findings of an earlier investigation."

The risk of wide dissemination of less than rigorous clinical science is not a new problem; however, the current COVID-19 pandemic has exacerbated the problem by precipitating the release of preliminary research findings that may be subsequently revised in the light of new evidence—or in some case proven to be completely wrong. This is further compounded by the recent establishment of non-peer-reviewed preprint servers which greatly lower the barrier to publishing and avoid critical peer review. Given the lack of effective vaccines and validated therapeutic choices against SARS-CoV-2, biomedical scientists are racing to publish suggestions for rapidly deployable therapeutic options. Although novel chemical and biologic entities are being evaluated as potential therapeutics for SARS-CoV-2 infections, the repositioning and off-label use of existing agents approved for unrelated conditions is widely advocated as a therapeutic approach against COVID-19 as it offers more rapid, actionable interventions against the virus. Off-label use allowed several groups to report potential efficacy of some agents in clinical studies, e.g. umifenovir[4], remdesivir[5,6] Table 1 summarizes some of the repositioning proposals undergoing clinical evaluation, many of which are investigator-initiated.

The hydroxchloroquinone saga

A case in point is the use of hydroxychloroquine (HCQ) and azithromycin in COVID-19. The first evidence for its potential came from an in-vitro study of a combination of remdesivir and chloroquine where growth inhibition of SARS-CoV-2 was reported. The original paper was submitted to *Nature Cell Research* on January 25, accepted on January 28 and published on February 4, following just 3 days in peer-review. This publication was followed by a Letter to Editor in *BioScience Trends*.

In early March, a medical group from IHU-Méditerranée Infection, Marseille, France used chloroquine (CQ), a related antimalarial drug used off-label for autoimmune diseases, such as systemic lupus erythematosus, to treat patients infected by SARS-CoV-2. The results of this open-label non-randomized clinical trial were submitted to *International Journal of Antimicrobial Agents* on March 16, accepted on March 17 and published on March 20. This report suggested that the chloroquine derivative HCQ, in combination with azithromycin, successfully clears SARS-CoV-2 infections. Upon close examination, however, this paper reported data from only 14 (single arm) and 6 (combination treatment with azithromycin to prevent bacterial superinfection) out of a total of only 26 patients. Despite the fact that the authors correctly mentioned the small sample size and very short follow up time, they nevertheless expressed their recommendation towards the application of both drugs as a curative and preventative therapy.

It was subsequently followed up by other trials and many publications in the lay press. These trials are currently inconclusive.The original study in March 2020 led, however, to an enormous level of activity and focus on HCQ, with serious ramifications across the industrial, medical, political and societal landscape.

Although rigorous, evidence-based results rooted in more than a century of experience in drug safety (the 'Pure Food and Drug Act' of 1906) are lacking, massive efforts have been focused on HCQ as a COVID-19 therapy, with global implications. Major multinational companies responding to requests from government leaders ramped up manufacturing of CQ and HCQ. Other countries have hoarded the products, while regulatory authorities are rushing through emergency approvals of the drugs without data on effective dosage or safety protocols. None of the related studies explicitly referred to existing posology and pharmacokinetic properties of the drugs (e.g., dosage, half-life, clearance etc.) that are essential for application guidance and approval in original indications.

The clinical community did not wait for the results of the more conclusive trials. And while the initial endorsement by the FDA was revoked in less than three months (*vide infra*), many self-medicating patients using and physicians prescribing HCQ ignoring or criticizing new study results [11] . As evidence of lack of efficacy of HCQ is published, further factors, such as the lack of zinc salts in combination with HCQ are raised to explain the negative results.

Lessons learned

First, the 24 hour peer-review process that led to the publication of the *International Journal of Antimicrobial Agents* paper was highly irregular. A rigorous, normal peer review process was not adopted. Had that occurred, it would have likely ensured that the publication that triggered this event would have had to reference and discuss a prior *failed* clinical study on HCQ published on March 6, 2020 in the *Journal of Zhejiang University*. In that controlled, open-label study based on 30 COVID-19 patients, half of which received HCQ. After seven days, 13 of the patients who were on the drug tested negative for the virus, compared with 14 of the people who on placebo. One of the patients on HCQ went on to develop severe illness, whereas the median recovery time taken was similar in both groups. The study concluded that HCQ is no more effective than the standard of care.

The article published by the group from IHU-Méditerranée Infection presents a peculiar case of a promising report with weak evidence and absence of proper peer review published in a respectable peer-reviewed journal instead of as a pre-print. Before this article (submitted on March 17) was published on March 20th, the US National press secretary announced on March 19 that HCQ had shown encouraging early results against COVID-19. This was followed by a blizzard of twitter- and TV-based communication claiming '100% cure' of COVID-19 by treating with HCQ, azithromycin and zinc sulfate without the need for hospitalization or ventilators. A Wuhan-based randomized clinical trial showed[13] shorter remission time and body temperature recovery time upon administration of HCQ in SARS-CoV-2 infected patients, while another study on 150 Chinese patients showed no difference in viral conversion rate.

On March 28, US Food and Drug Administration (FDA), but not the European Union, granted Emergency Use Authorization (EUA) for both chloroquine and HCQ for certain hospitalized COVID-19 patients. The FDA officially revoked the EUA on June 15, after a series of addendum warnings about HCQ side effects, especially the potential for fatal arrhythmia due to QTc prolongation, as well as other cardiac events. Despite additional evidence gathered over the past months, enforcing the non-existing benefit of HCQ in prophylactic setting, there is still advocacy for self-medicated prophylactic usage. Neither the study published by the medical group from the IHU-Méditerranée Infection, nor forthcoming studies embracing HCQ for COVID-19, were reproducible. Despite scientific weakness, they became the object of political attention causing global shortage of the drugs that affected auto-immune disease patients with legitimate therapeutic needs.

The two initial HCQ reports bypassed existing scientific checks and balances, highlighting the importance of sound peer-review, which should be observed when disclosing novel therapies, in particular facing a global crisis. It is important to ensure that scientific publications undergo rigorous peer review, even in times of emergency, remains crucial. Moreover, *Primum non nocere* (First, do no harm), a central tenet of medical practice, was compromised because 1) patients with legitimate medical need (e.g., rheumatoid arthritis) for HCQ could no longer procure the drug; and 2) those patients who did receive the HCQ/azithromycin combination gained no benefit but were exposed to the therapy's increased risk of cardiovascular mortality.

What to do about misinformation?

It is critical we learn from the HCQ experience as we look for immediate medical solutions to this crisis. The COVID-19 pandemic has resulted in an unprecedented number of other therapeutic proposals, from peer-reviewed and pre-print publications, to blog posts, tweets, TV and other communication channels. No matter how rational or sound, this many proposals cannot be systematically evaluated and prioritized by any single group, institution, or regulator. There is a clear need to understand the ever-growing stream of data, information and knowledge being published, to collate, process and structure it in real time.

In this respect, dictionary-based text mining, coupled with specialized artificial intelligence/machine learning, originally called statistical pattern recognition, such as BioBERT, a bidirectional biomedical language representation model can help achieve that potential. Rigorous, evidence-based peer review coupled with open data and computer-aided technologies offer a way out of the current dilemma and provide the opportunity to make reasoned scientific breakthroughs in a crisis. Although preprint services allow rapid dissemination of new findings, they are not peer-reviewed and can easily mislead non-experts and encourage sensationalist and erroneous public coverage. AI/ML could provide an interpretable score that can be deconvoluted into the most important features. This score could be published on a preprint server and sent to the regulator should it discover fraud

Clinical drug development requires multiple, distinct areas of expertise. Unbiased clinical data, compiled in real time, ought to be made accessible without restrictions, and intellectual property rights should be waived for this, and future pandemics. Although coordinated efforts are on-going, this pandemic offers a unique opportunity to lay the foundation for synchronized global workflows that will ensure data veracity, provide an unbiased and multi-viewpoint assessment of therapeutic alternatives, and allow efficient allocation of computational, human and experimental resources. This must occur in the context of allowing peer-review and fact-checking and incorporation of relevant domain expertise.

Traditionally, such activities would take place in laboratories and be followed by human clinical trials. Given the almost complete shutdown of animal research facilities and need for focused rational clinical trials, it is worthwhile to explore how many of these activities can be supplemented or even replaced by the capabilities that are now available through *in silico* technologies, such as machine learning, systems biology and computer-aided drug repurposing. These technologies have matured in recent years and are ready to become an integral part of the global workflow, to prioritize novel drug targets and new chemical entities as well as to evaluate off-label/drug repositioning proposals. Such workflows—based for example on Drug Repositioning Evidence Level (DREL) —could be used to evaluate drug repositioning candidates. By integrating multiple layers of data, information and knowledge, and processing the massive stream of repositioning proposals, validated machine intelligence-based methods could serve, in the near future, as a decision support system for policymakers, healthcare providers and society at large. If properly resourced and implemented, such a synchronized workflow could assist in

assembling disparate evidence and hypotheses into actionable healthcare solutions to tackle the current and future inevitable pandemics.

In practice, the deployment of AI/ML methods requires a comprehensive understanding of their advantages and weaknesses. AI/ML is powerful to identify relevant patterns within large set of nonlinear data without the need for manual feature engineering as they can learn implicit rules from the data provided. While the amount of data needed to train such algorithms might be an issue, the ability of AI/ML to make sense of large amount of data is an advantage in many circumstances. For instance, the Smith–Waterman algorithm and Pfam are standard methods for the prediction of protein functions but they are not fast enough to handle large number of protein sequences. AI/ML offers alternatives to address both issues. For instance, method DeepFam is an alignment-free method extracting functional information from sequences without requiring multiple sequence alignments. When compared to the state-of-the-art methods, DeepFam performed better in terms of accuracy and runtime for predicting protein functions. In this context, the emergence of AI/ML approaches is incremental and can be built upon classical sequence similarity and genome analysis with tools such as BLAST.

The point where traditional algorithmic analysis becomes AI is also moot. In general, AI/ML comes into its own with large datasets, where models have non-linear outputs and complex descriptor datasets are in use—precisely the type of situation found during the pandemic response. Specifically, although a simple sequence similarity search would allow the comparison of a new pathogen sequence to prior art (existing pathogen sequences) and the selection of the closest relative (and therefore best candidate for simple transitive knowledge transfer), performance can be boosted by the identification of key binding site residues and consideration of the impact of sequence changes on protein function and drug binding in real time.

Machine learning is already starting to be used to identify biological targets for therapeutic intervention in heterogeneous disease[26] and find suitable drug candidates that bind those targets (Table 2). Similarly, if empirical clinical observations in a paper are used to propose a drug as a potential treatment approach, machine learning could and should be used to rapidly simulate efficacy and side effects in (preferably stratified) populations. A synchronized workflow using machine learning methods could be based on resources available for analyzing targets, drugs and related potential side effects such as the Side Effect Resource (SIDER) that combines data on drugs, targets and side effects recorded during clinical trials and the FDA Adverse Event Reporting System (FAERS) that gives access to adverse event reports and medication error reports previously submitted to FDA.

There are still several unknowns about the biology and mode of action of SARS-CoV-2 virus. However, information about the sequence of the viral genome and discovery of receptors used by the virus to infect cell and knowledge of structure of the virus allow identifying potential targets for direct acting antivirals. As data has emerged on population scale pathology, it has become clear that an over-active host immune response is a clear driver of more serious disease. Naturally, the data gathering, analytics strategies and focus for therapy discovery have responded to these data.

Furthermore, machine learning algorithms can accelerate the design of clinical trials by automatically identifying suitable subjects, ensuring the correct distribution to groups of study

participants and providing an early warning system for a clinical trial that is not producing meaningful results. Computational drug repurposing accelerates the drug development process and reduces the associated costs. To identify the right repurposing candidates, it is important to identify known molecular targets, to predict novel molecular targets for known drugs, and to consider dosing, pharmacokinetic and safety-related parameters. With its ability to analyze millions of examples of drug and patient data to generate hypotheses and then provide evidence supporting or challenging them, machine learning can be used for identifying new indications for already known drugs and for combining existing drugs in ways that give them therapeutic powers that each lacks in isolation.

Within healthcare and drug discovery, AI/ML should be implemented as discrete components in current human workflows; as opposed to the attempted or perceived complete replacement of human control. Integration of AI/ML offers powerful alternatives but can only be successful with multidisciplinary teams involved in the design AI/ML solutions adapted to each situation. From this viewpoint, human expertise and final decision making will remain essential. There are currently too few examples of large-scale integration of AI/ML technologies and in drug discovery where the time frame for a drug to undergo preclinical testing and clinical trials is especially long, more time is needed to assess their real impact.

AI/ML algorithms already deployed within the drug development pipeline have greatly improved. For instance, the synthetic tractability that was a weakness of the first AI/ML *de novo* design methods can now be evaluated using synthetic accessibility scores. When properly designed in collaboration with medicinal chemist experts, platforms for *de novo* design can prioritize synthetically tractable molecular structures with the desired biological activity. Moreover, state-of-the-arts AI-based methods for *de novo* design can generate molecular structures using restricted information. Binding site amino acid environment and co-crystallized fragment for instance provide the pocket and ligand features needed to perform either ligand-based or pocket-based generation. Nevertheless, challenges encountered when developing AI/ML solutions for *de novo* molecular generation or for medical imaging prognosis[27] also demonstrated that there is a need for developing and improving reporting standards and metrics as well as best practices for data sharing, requirement for algorithm availability which should be adapted to the strict requirements and expectations of medical sciences and healthcare.

Conclusions

The COVID-19 pandemic has highlighted the need for new tools to complement existing peer-review mechanisms for ensuring the veracity and robustness of the biomedical information published to guide clinical practice and shape public health policy. It has also shown that the research community is capable of generating large amounts of heterogeneous data in a short period of time—information on a scale and speed that can confound human interpretation. In this context, we believe that machine intelligence has a critical role to play in bolstering data to supplement peer-reviewed papers. We need to bring these tools immediately to focus on this global problem.

“The aging related ICD-11 codes are the basis for Longevity Medicine”
(Aging as a disease)

The rapidly evolving field of Longevity Medicine focuses on identifying individual ageing trajectories by determining the biological age over time and targeting ageing mechanisms to extend healthspan, the time lived with an optimal performance and health. Currently, the ageing trajectory is determined by reaching an individual’s optimal peak performance (OPP), followed by an individually variable, but overall physical and mental decline. (Fig.1) The latter is often accepted as an inevitability, accompanied by age-related diseases, multimorbidity, frailty, and multisystemic failure.

At different states of the personal life cycle, predominantly reactive medical systems accommodate individuals and capture limited physical and mental data, rarely collected during younger adulthood.

Longevity Medicine provides an ecosystem for collecting, analyzing, and translating longitudinal data, drawing conclusions from the momentary state of health towards predictive and prognostic trajectories, aiming at mitigating and eliminating specific risks of morbidity. The ultimate goal is to bring the individual closer to the state of OPP during the entire lifespan. (Fig.1)

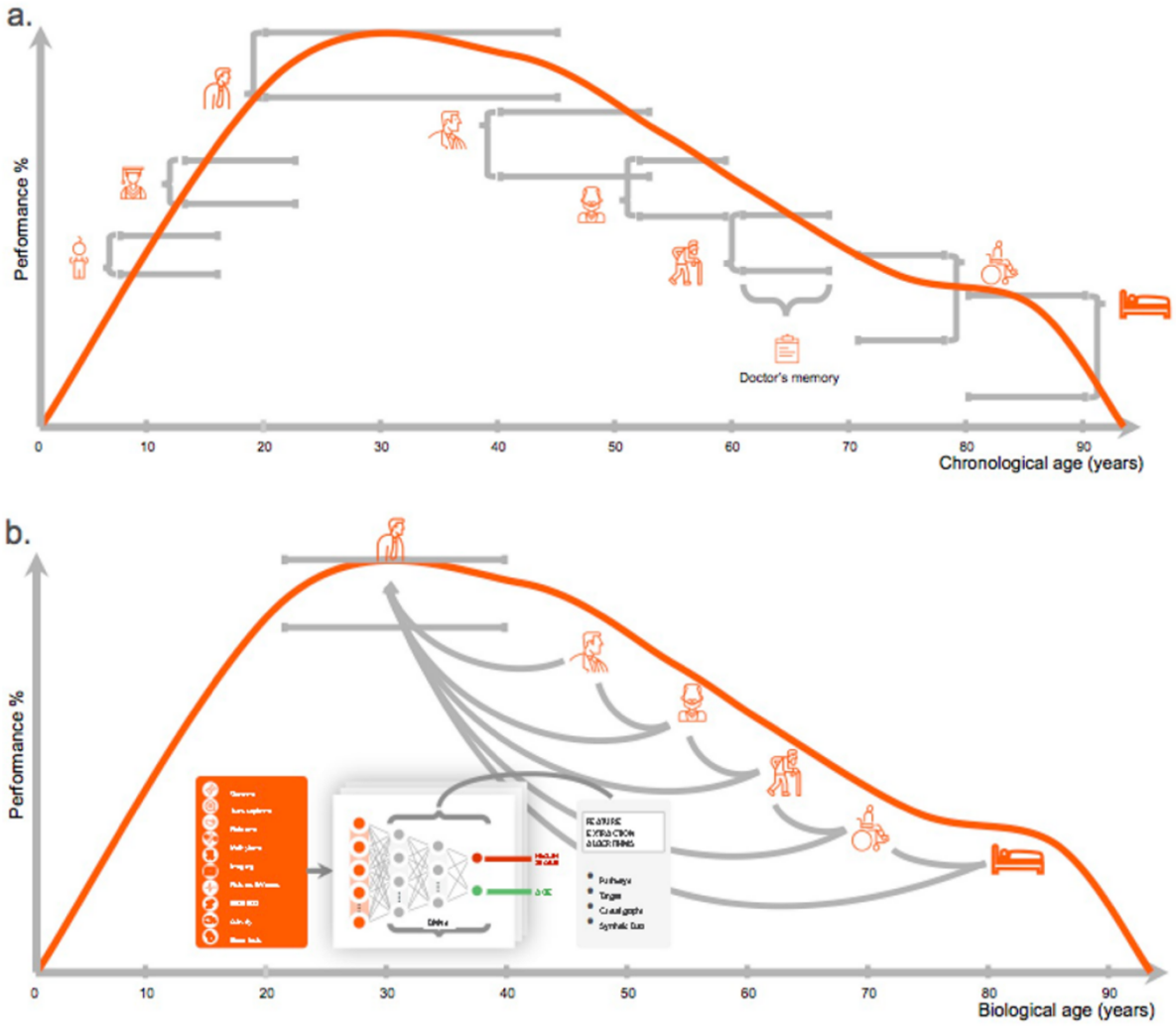


Figure 1. Traditional vs. Longevity Medicine. a. The current preventive medicine evaluates the patient within the reference range for the patient's age group and is aiming to optimize the parameters in the context of the patient's age group. b. Longevity Medicine uses AI to evaluate the individual's biological age throughout the course of life, looking for ways to to reduce the gap between the current parameters and the parameters of maximum physical performance for the ideal biological age to maintain and prolong the individual peak performance (IPP). Longevity medicine identifies ways to restore the current biological age to the desired biological age.

The ICD11 supports installed new dynamics in the nascent field of Longevity Medicine by classifying aging as a disease. It allows physicians to target aging in a comprehensive rather than a less efficacious disease and syndromes-oriented manner. Banerjee et al. called for excluding old age from ICD-11, suggesting replacement by frailty.

Whether the term "old age" is the best choice terminology for a state of multi-malfunction is a semantic, redundant debate. Firstly, ICD-codes are carefully considered and revised before being implemented. Secondly, frailty refers to, mostly but not exclusively, age-related disabilities, while

old age is not always associated with frailty. Thus these terms are not mutually exclusive and can co-exist in the ICD, as a part of a hierarchy of causation. The code, XT9T, guarantees coding for measurable age-related processes, e.g. inflammaging, mitochondrial dysfunctions, etc. The MG2A code, on the other hand, is representative of the paradigm shift in the definition of an individual's age, from chronological to biological, and will promote the development of therapies to optimize biological age. This paradigm shift in the definition of age, along with technological advances in the ability to control biological age, has led to considerable investment in the field of Longevity to develop interventions targeting aging mechanisms and systemic rejuvenation rather than a single organ or system at a time.

Thus, the current ICD-classification should remain, to recognize and foster the rapid development of Longevity Medicines and to allow physicians treat biological age rather than specific diseases, thereby extending the healthspan and lifespan beyond current approaches.

Part IX. Discussion and conclusions

Discussion

Cardiongevity and Senocardiology

The use of biological age clocks in cardiology has emerged as a promising approach for assessing cardiovascular health and predicting disease risk. Biological age clocks are tools that measure an individual's biological age, which is a reflection of their overall health and aging process, rather than their chronological age. By incorporating various biomarkers and genetic information, these clocks can provide a more accurate assessment of an individual's risk for cardiovascular diseases compared to traditional risk factors alone.

The integration of longevity medicine approaches into cardiology has the potential to revolutionize the field and improve patient outcomes. Longevity medicine focuses on understanding and modulating the underlying mechanisms of aging to promote healthier and longer lives. By incorporating this approach into cardiology, clinicians can not only diagnose and treat cardiovascular diseases but also address the root causes of aging-related cardiovascular decline. This integrated approach allows for a more comprehensive and personalized management of patients, targeting both the prevention and treatment of cardiovascular diseases and age-related comorbidities.

The impact of integrating longevity medicine into cardiology extends beyond disease management. It emphasizes a proactive and preventive approach, encouraging individuals to adopt healthier lifestyles, such as regular exercise, balanced diet, stress reduction, and adequate sleep. By promoting these lifestyle modifications, cardiovascular health can be optimized, and the risk of developing age-related diseases can be significantly reduced.

Furthermore, the integration of longevity medicine into cardiology promotes a shift from a disease-centered model to a patient-centered model. It recognizes the importance of individualized care, considering not only the patient's cardiovascular health but also their overall well-being and quality of life. This holistic approach takes into account factors such as mental health, social support, and functional status, aiming to improve not just the quantity but also the quality of life for patients.

Sex and gender in cardiology

Cardiology has increasingly recognized the importance of considering sex and gender differences in cardiovascular health. Sex refers to the biological and physiological characteristics that distinguish males from females, while gender refers to the socially constructed roles, behaviors, and expectations associated with being male or female. Research has shown that sex and gender can influence various aspects of cardiovascular disease, including risk factors, symptoms, diagnosis, treatment response, and outcomes.

Men and women may exhibit differences in the prevalence, presentation, and progression of cardiovascular conditions. For example, men tend to have a higher incidence of coronary artery disease at a younger age, while women may experience more atypical symptoms or present with microvascular disease. Hormonal factors, such as estrogen, have been suggested to play a protective role in women before menopause, contributing to differences in the development and manifestation of certain heart conditions.

Recognizing these sex and gender differences is crucial for providing effective and personalized care in cardiology. It allows for tailored risk assessment, early detection, and appropriate management strategies based on an individual's specific characteristics. Furthermore, considering gender-related factors, such as social determinants of health, lifestyle patterns, and healthcare-seeking behaviors, helps address disparities in cardiovascular outcomes between different populations.

In recent years, guidelines and recommendations have been developed to highlight the importance of sex and gender considerations in cardiology research, clinical practice, and public health efforts. These initiatives aim to improve the understanding of sex-related biological mechanisms, address gaps in knowledge, and promote equitable access to cardiovascular care for all individuals.

Cardiooncology and geroncology

Cardiooncology is an emerging field that focuses on the intersection between cardiology and oncology, recognizing the cardiovascular complications and implications of cancer treatments. As advancements in cancer therapies have led to improved survival rates, the long-term cardiovascular health of cancer patients has become a critical consideration.

Cancer treatments, such as chemotherapy, targeted therapies, radiation, and immunotherapy, can have direct and indirect effects on the cardiovascular system. Some treatments may cause cardiotoxicity, leading to conditions such as cardiomyopathy, heart failure, arrhythmias, and vascular diseases. Additionally, cancer patients may have pre-existing cardiovascular conditions that require careful management during their cancer treatment journey.

The field of cardiooncology aims to provide comprehensive cardiac care to cancer patients, from risk assessment and prevention to monitoring and treatment of cardiovascular complications. It involves a multidisciplinary approach, bringing together cardiologists, oncologists, radiologists, and other specialists to collaborate in the care of these patients. Close monitoring of cardiac function, early detection of cardiotoxicity, and timely interventions are key components of cardiooncology practice.

Cardiooncology also plays a vital role in optimizing cancer treatment strategies. By identifying potential cardiovascular risks before initiating cancer therapies, healthcare providers can tailor treatment plans to minimize the impact on the heart and blood vessels. This approach ensures that cancer patients receive effective treatment while also safeguarding their cardiovascular health.

Conclusions

In conclusion, the utilization of biological age clocks in cardiology and the integration of longevity medicine approaches have the potential to transform the field by providing a more accurate assessment of cardiovascular health and addressing the underlying mechanisms of aging. This integrated approach promotes preventive measures, personalized care, and improved patient outcomes, ultimately contributing to a healthier and longer life for individuals at risk of cardiovascular diseases.

Integrating sex and gender considerations in cardiology is essential for a comprehensive understanding of cardiovascular disease and its management. By recognizing and addressing the unique aspects of sex and gender differences, healthcare providers can deliver more personalized and effective care, ultimately leading to improved cardiovascular outcomes for both men and women.

The field of cardiooncology is rapidly evolving, with ongoing research and clinical trials focused on understanding the mechanisms of cardiotoxicity, developing cardioprotective strategies, and improving long-term cardiovascular outcomes for cancer survivors. By integrating the expertise of cardiologists and oncologists, cardiooncology aims to provide comprehensive care that addresses both the cancer and cardiovascular needs of patients, ultimately enhancing their overall well-being and quality of life.

List of abbreviations

CVD: Cardiovascular disease
AI: artificial intelligence
BMI: Body Mass Index
ML: machine learning
ECG: electrocardiogram
CNN: convolutional neural network
PCPF: post-COVID pulmonary fibrosis
DNNs: deep neural networks
DAC: Deep Aging Clocks
GHDDI: the Global Health Drug Discovery Institute
TAME:the Targeting Aging with Metformin
NCD: non-communicable
HIFs: hypoxia-inducible factors
BCSCs: breast cancer stem cells
mTOR:mammalian target of rapamycin
TICs: tumor-infiltrating cells
TAMs: tumor-associated macrophages
VEGF: vascular endothelial growth factor
ECM: extracellular matrix
MMPs: matrix metalloproteinase
ECs: endothelial cells
NuFF: neonatal fibroblasts
Tif1γ:transcription intermediary factor 1γ
TGF-β: transforming growth factor -β
EMT:epithelial-mesenchymal transition
LOX: lysyl oxidase
MSC: mesenchymal stem cell
A2BR: adenosine receptor 2B
qBOLD: quanti tative blood oxygenation level dependent
VAM: vascular architectural mapping
TNBCs: triple-negative breast cancers
mBC: Male breast cancer
fBC: female breast cancer
SEER:Surveillance, Epidemiology, and End Results
DFS:disease free survival
OD:optical density
EFIM: the European Federation of Internal Medicine
IMAGINE: the Internal Medicine Assessment of Gender differences in Europe
SD: standard deviation
ESMO: the European Society for Medical Oncology
CSCO: the Chinese Socmpoiety of Clinical Oncology
CACA: China Anti-Cancer Association
CSMO: Chinese Symposium on Medical Oncology
SC: scientific committee

EVD: ebola viral disease
MP: methylprednisolone
CA: cardiac arrest
CPR: cardiopulmonary resuscitation
VF: ventricular fibrillation
PC: precordial compression
CPP: coronary perfusion pressure
ANOVA: analysis of variance
MAP: mean arterial pressure
SDF: Sidestream Dark-Field
IL-6: Interleukin 6
PCI: percutaneous *coronary* intervention
LAD: left anterior descending coronary artery
LVEDD: left ventricular end-diastolic diameter
LVESD: left ventricular end-systolic diameter
LVFS: left ventricular fractional shortening
LVEF: left ventricular ejection fraction
MPO: myeloperoxidase
ROS: reactive oxygen species
IHC: immunohistochemistry
TNF-α: tumor necrosis factor-α
ROSC: return of spontaneous circulation
MPI: myocardial performance index
CQ: chloroquine
FDA: Food and Drug Administration
EUA: Emergency Use Authorization

List of publications

Time frame of the Cardiopath program: 2020-2023
First Author: 28
Last Author: 10

· *"Artificial intelligence in longevity medicine"*. Zhavoronkov A, **Bischof E**, Lee K-F. Nature Aging. 2021;1(1):5-7.

· *"Advanced pathological ageing should be represented in the ICD"*. **Bischof E**, Maier AB, Lee K-F, Zhavoronkov A, Sinclair D. The Lancet Healthy Longevity. 2022;3(1):e12.

· *"Sex-disaggregated data in COVID-19 vaccine trials"*. Vijayasingham L, **Bischof E**, Wolfe J. The Lancet. 2021;397(10278):966-7.

· *"AI in Longevity Medicine"*. Radenkovic D, Zhavoronkov A, **Bischof E**. Artificial Intelligence in Medicine. 2021.

· *"Increased pace of aging in COVID-related mortality"*. Galkin F, Parish A, **Bischof E**, Zhang J, Mamoshina P, Zhavoronkov A. Life. 2021;11(8):730.

· *"Effects of Methylprednisolone on Myocardial Function and Microcirculation in Post-resuscitation: A Rat Model"*. Wang C, **Bischof E**, Xu J, Guo Q, Zheng G, Ge W, Hu J, Margarint ELG, Bradley JL, Peberdy MA. Frontiers in Cardiovascular Medicine. 2022;9.
· *"Prevention and Control Strategies Based on the Epidemiology of Imported COVID-19 in Mainland China"*. Han N, Li Q, Wang T, Han J, Chen S, Morgan R, Liu Z, **Bischof E**, Naples VP. The Lancet. 2020.

· *"The potential of rapalogs to enhance resilience against SARS-CoV-2 infection and reduce the severity of COVID-19"*. **Bischof E**, Siow RC, Zhavoronkov A, Kaeberlein M. The Lancet Healthy Longevity. 2021;2(2):e105-e11.

· *"Longevity medicine: upskilling the physicians of tomorrow"*. **Bischof E**, Scheibye-Knudsen M, Siow R, Moskalev A. The Lancet Healthy Longevity. 2021;2(4):e187-e8.

· *"Management of Elderly and Old Cancer Patients-Geriatric Oncology"*. **Bischof E**, Hurni B, Vetter M. healthbook TIMES Oncology Hematology. 2021;10(4):28-33.

· *"Sex and gender differences in Alzheimer's disease: current challenges and implications for clinical practice: Position paper of the Dementia and Cognitive Disorders Panel of the European Academy of Neurology"*. Ferretti M, Martinkova J, **Biskup E**, Benke T, Gialdini G, Nedelska Z, Rauen K, Mantua V, Religa D, Hort J. European journal of neurology. 2020;27(6):928-43.

· *"Towards precision medicine: inclusion of sex and gender aspects in COVID-19 clinical studies—acting now before it is too late—a joint call for action"*. **Bischof E,** Oertelt-Prigione S, Morgan R,

Klein SL, (SGC) SaGiCCTWG, Group GaCW. International journal of environmental research and public health. 2020;17(10):3715.

· *"Clinical trials for COVID-19 should include sex as a variable"*. **Bischof E**, Wolfe J, Klein SL. The Journal of clinical investigation. 2020;130(7):3350-2.

· *"Towards precision medicine: inclusion of sex and gender aspects in COVID-19 clinical studies—acting now before it is too late—a joint call for action"*. **Bischof E**, Oertelt-Prigione S, Morgan R, Klein SL, (SGC) SaGiCCTWG, Group GaCW. International journal of environmental research and public health. 2020;17(10):3715.

·· *"Stress, diet, exercise: Common environmental factors and their impact on epigenetic age"*. Galkin F, Kovalchuk O, Koldasbayeva D, Zhavoronkov A, **Bischof E**. Ageing Res Rev. 2023;88:101956. [published online ahead of print, 2023 May 19].

· *"Growth differentiation factor-15 and all-cause mortality in patients with suspected myocardial infarction"*. Walter J, Nestelberger T, Boeddinghaus J, Twerenbold R, Croton L, Badertscher P, Wildi K, Wussler D, **Biskup E**, de Lavallaz JdF, Mueller C. International journal of cardiology. 2019;292:241-5.

· *"Towards AI-driven longevity research: An overview"*. Marino N, Putignano G, Cappilli S, Chersoni E, Santuccione A, Calabrese G, **Bischof E**, Vanhaelen Q, Zhavoronkov A, Scarano B. Frontiers in Aging. 2023; 4:1057204.

· *"Mitigating COVID-19 Mortality and Morbidity in China's Aging Population: A Focus on Available Medications and Future Developments"*. **Bischof E**. AGING AND DISEASE. 2023.

· *"Organization of Protective Regime for Elderly Population in the COVID-19 Pandemic Period"*. Yakushin MA, Gorenkov RV, Vasilieva TP, Melerzanov AV, Alexandrova OY, Yarotskiy SY, Malakhova AR, Moskalev A, **Bischof E**. Journal of Environmental Science and Public Health. 2022;5:490-503.

· *"Application of the PHENotype SIMulator for rapid identification of potential candidates in effective COVID-19 drug repurposing"*. Maria NI, Rapicavoli RV, Alaimo S, **Bischof E**, Stasuzzo A, Broek JA, Pulvirenti A, Mishra B, Duits AJ, Ferro A. Heliyon. 2023;9(3).

· *"Hormone Receptor Expression in Primary and Recurrent High-Grade Serous Ovarian Cancer and Its Implications in Early Maintenance Treatment"*. Vetter M, Stadlmann S, **Bischof E**, Georgescu Margarint EL, Sch?tzau A, Singer G, Heinzelmann-Schwarz V, Montavon C. International Journal of Molecular Sciences. 2022;23(22):14242.

· *"Identification of Therapeutic Targets for Amyotrophic Lateral Sclerosis Using PandaOmics–An AI-Enabled Biological Target Discovery Platform"*. Pun FW, Liu BHM, Long X, Leung HW, Leung GHD, Mewborne QT, Gao J, Shneyderman A, **Bischof E**, Ozerov IV, Wang J. Frontiers in Aging Neuroscience. 2022;14:638.

· *"Meeting report: Aging research and drug discovery"*. Meron E, Thaysen M, Angeli S, Antebi A, Barzilai N, Baur JA, Bekker-Jensen S, Birkisdottir M, **Bischof E**, Bruening J. Aging (Albany NY). 2022;14(2):530.

· *"Potential Clinical Applicability of The PHENotype SIMulator for In Silico Viral Co-Infection Studies in COVID-19"*. Maria NI, Rapicavoli RV, Alaimo S, **Bischof E**, Pulvirenti A, Mishra B, Duits AJ, Ferro A. The Journal of Immunology. 2022;208(1_Supplement):172.19-.19.

· *"Awareness of sex and gender dimensions among physicians: the European federation of internal medicine assessment of gender differences in Europe (EFIM-IMAGINE) survey"*. **Biskup E**, Marra AM, Ambrosino I, Barbagelata E, Basili S, de Graaf J, Gonzalvez-Gasch A, Kaaja R, Karlafti E, Lotan D. Internal and emergency medicine. 2022;17(5):1395-404.

· *"Adherence to bone health guidelines in patients with hormone receptor-positive early breast cancer: Status and clinical impact in a Swiss cohort experience"*. **Bischof E**, Schwab FD, Margarint ELG, Montavon C, Zünti I, Schollbach A, Sch?tzau A, Hirschmann A, Landin J, Meier C. Bone Reports. 2022;16:101160.

· *"Longevity Foundation: Perspective on Decentralized Autonomous Organization for Special-Purpose Financing"*. **Bischof E**, Botezatu A, Jakimov S, Suharenko I, Ostrovski A, Verbitsky A, Yanovich Y, Zhavoronkov A, Zmudze G. IEEE Access. 2022;10:33048-58.

· *"Hypoxia in Breast Cancer—Scientific Translation to Therapeutic and Diagnostic Clinical Applications"*. Zhang Y, Zhang H, Wang M, Schmid T, Xin Z, Kozhuharova L, Yu W-K, Huang Y, Cai F, **Biskup E**. Frontiers in Oncology. 2021;11:652266.

· *"Management of Colorectal Cancer Patients During The COVID-19 Pandemic: Triage and Treatment"*. Xiang P, Han J, Leung TI, Maria NI, Li P, **Biskup E**. Times of hematology and oncology 2021.

· *"Sleep disorders and dementia"*. Nasta B, Hill-Strathy M, **Biskup E**, Rauen K. Sex and Gender Differences in Alzheimer's Disease. 2021:207-32.

· *"Organization of Protective Regime for Elderly Population in the COVID-19 Pandemic Period"*. Mikhail A Yakushin RVG, Tatiana P Vasilieva, Alexander V Melerzanov, Oxana Yu Alexandrova, Sergei Yu Yarotskiy, Aleksandra R Malakhova, Alexey Moskalev, **Evelyne Yehudit Bischof**. J Environ Sci Public Health. 2021(2021//5 (4)):490-503.

· *"Rapid identification of druggable targets and the power of the phenotype simulator for effective drug repurposing in COVID-19"*. Maria NI, Rapicavoli RV, Alaimo S, **Bischof E**, Stasuzzo A, Broek JA, Pulvirenti A, Mishra B, Duits AJ, Ferro A. Research Square. 2021.

· *"Anthracycline-induced cardiotoxicity in the chemotherapy treatment of breast cancer: Preventive strategies and treatment"*. Cai F, Luis MAF, Lin X, Wang M, Cai L, Cen C, **Biskup E**. Molecular and clinical oncology. 2019;11(1):15-23.

· *"Dance of the Porcupines: A View into the Rapid Development of Covid-19 Vaccines from Around the World—Part 1"*. Levin J, **Bischof E**, Prewitt E. NEJM Catalyst Innovations in Care Delivery. 2021;2(1).

· *"In vivo and in situ real-time fluorescence imaging of peripheral nerves in the NIR-II window (vol 12, pg 3059, 2019)"*. Feng Z, Yang Y, Zhang J, Wang K, Li Y, Xu H, Wang Z, **Biskup E**, Dong S, Yang X. NANO RESEARCH. 2021.

· *"A phase I study of the safety and activity of K-001 in patients with advanced pancreatic ductal adenocarcinoma"*. Cui J, Yang H, Liu J, Chen D, Hu J, Zhang H, **Biskup E**, Wang Y, Han T, Mao T, Jiao F. BMC cancer. 2021;21(1):672.

· *"A Phase I Study of K-001 in Patients with Advanced Pancreatic Ductal Adenocarcinoma"*. Cui J, Yang H, Liu J, Chen D, Hu J, Zhang H, **Biskup E**, Wang Y, Han T, Mao T, Jiao F. 2021.

· *"Oncofertility Knowledge and Communication: Comparison Between Medical and Surgical Oncologists and Breast Cancer Patients in Academic Chinese Centers"*. **Biskup E**, Xin Z, Li R, Zucal JP, Lu Y, Sun Y, Appiah LC, Lindheim SR, Zhang H. Frontiers in Surgery. 2021:361.

· *"Pertuzumab As Second-or Later-Line Therapy For Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer: A Swiss Clinical Experience"*. **Biskup E**, Sartorius CM, Müller A, Leo C, Nussbaum CU, Margarint ELG, Koychev D, Schreiber A, Taverna C, Thorn D. 2021.

· *"Sex differences in psychiatric disorders and their implication for dementia"*. **Biskup E**, Jordan V, Nasta B, Rauen K. Sex and Gender Differences in Alzheimer's Disease. 2021:187-206.

· *"p-CREB-1 at Ser 133 is a potential marker for breast cancer"*. Xin Z, Hu H, Lao Z, Zhu L, **Biskup E**, Zhang H. Eur Rev Med Pharmacol Sci. 2020;24(22):11628-38.

· *"Will repeated ablative Er: YAG laser treatment sessions cause facial skin sensitivity? Results of a 12-month, prospective, randomized split-face study"*. Wang X, Yuan C, Wo Y, Qian L, Liu K, Zhang Z, Zhang Y, Xu H, Chen X, **Biskup E**. Rejuvenation Research. 2020;23(2):122-9.

· *"Internet cognitive behavioral therapy with or without face-to-face psychotherapy: a 12-weeks clinical trial of patients with depression"*. Rauen K, Vetter S, Eisele A, **Biskup E**, Delsignore A, Rufer M, Weidt S. Frontiers in Digital Health. 2020:4.

· *"Internet cognitive behavioral therapy with or without face-to-face psychotherapy in patients with depressive disorders: a longitudinal clinical trial over 12 weeks"*. Rauen K, Vetter S, Eisele A, **Biskup E**, Delsignore A, Rufer M, Weidt S. Frontiers in Digital Health. 2020;2:4.

· *"Pandemics and violence against women and children"*. Peterman A, Potts A, O'Donnell M, Thompson K, **Biskup E**, Shah N, Oertelt-Prigione S, Van Gelder N. 2020.

· *"Starvation stress attenuates the miRNA-target interaction in suppressing breast cancer cell proliferation"*. Lü J, Zhang C, Han J, Xu Z, Li Y, Zhen L, Zhao Q, Guo Y, Wang Z, **Bischof E**. BMC cancer. 2020;20:627.

· *"A new territory and its pioneer: opening up a dominant research stream for a translational research area"*. Liu Y, **Biskup E**, Wang Y, Cai F, Zhang X. Scientometrics. 2020;125:1213-28.

· *"Artificial intelligence, drug repurposing and peer review"*. Levin JM, Oprea TI, Davidovich S, Clozel T, Overington JP, Vanhaelen Q, Cantor CR, **Bischof E**, Zhavoronkov A. Nature Biotechnology. 2020;38(10):1127-31.

· *"Facilitating credentialing and engagement of international physician-migrants during the COVID-19 crisis and beyond"*. Leung TI, **Biskup E**, DeWitt D. Rural and Remote Health. 2020;20(3).

· *"AFTER COVID PEAKS: VIEWS FROM FOUR GLOBAL REGIONS"*. Leung TI, Ali Y, **Bischof E**, Belaustegui AS, Frank MG. Society of General Internal Medicine. 2020.

· *"Sepsis at ICU admission does not decrease 30-day survival in very old patients: a post-hoc analysis of the VIP1 multinational cohort study"*. Ibarz M, Boumendil A, Haas LE, Irazabal M, Flaatten H, De Lange DW, Morandi A, Andersen FH, **Bischof E**, Bertolini G, Cecconi M. Annals of intensive care. 2020;10(1):56.

· *"Pulmonary embolism: a practical guide for the busy clinician"*. Demelo-Rodriguez P, Galeano-Valle F, Salzano A, **Biskup E**, Vriz O, Cittadini A, Falsetti L, Ranieri B, Russo V, Stanziola AA. Heart failure clinics. 2020;16(3):317-30.

· *"Abstract P1-17-05: Oncofertility awareness and attitudes among health care providers and patients in a Chinese academic setting: Preliminary results"*. **Biskup EM**, Zhaochen X, Appiah LC, Sun Y, Lu Y, Zhang H, Lindheim SR. Cancer Research. 2020;80(4_Supplement):P1-17-05-P1-17-05.

· *"Abstract P4-17-09: Clinicopathological and prognostic significance of preoperative PGC-1alpha plasma levels in male breast cancer patients"*. **Biskup EM**, Cai F, Lindheim S. Cancer Research. 2020;80(4_Supplement):P4-17-09-P4-17-09.

· *"Clinicopathological and prognostic significance of preoperative PGC-1alpha plasma levels in male breast cancer patients"*. **Biskup EM**, Cai F, Lindheim S. CANCER RESEARCH. 2020;80(4).

· *"Gender balance at oncology conferences in China"*. **Biskup E**, Xin Z, Khanna K, Leung TI, Zhang H. The Lancet Oncology. 2020;21(9):1138-40.

· *"Medicine in China–Balancing the Progress and the Foundations in Medical Education"*. **Biskup E**, Wo Y. Praxis. 2020.

· *"Fighting diagnostic and therapeutic nihilism in the elderly with cancer"*. **Biskup E**, Vetter M, Wedding U. Ann Palliat Med. 2020;9(3):1324-32.

· *"Sex, gender, and precision medicine"*. **Biskup E**, Raparelli V, Leung TI. JAMA internal medicine. 2020;180(8):1128-9.

· *"Looking to the future to prepare for Covid-19's second wave"*. **Biskup E**, Prewitt E. NEJM Catalyst Innovations in Care Delivery. 2020;1(3).

· *"Gender medicine: Towards a gender-specific treatment of neuropsychiatric disorders"*. **Biskup E**, Martinkova J, Ferretti MT. Handbook of clinical neurology. 2020;175:437-48.

· *"Doctors' Made in China'–Locally Trained, Globally Excellent"*. **Biskup E**, Li F, Dong S, Wo Y. Praxis. 2020.

· *"Understanding COVID-19 new diagnostic guidelines–a message of reassurance from an internal medicine doctor in Shanghai"*. **Bischof E**, Chen G, Ferretti MT. Swiss medical weekly. 2020;150.

· *"Understanding COVID-19 new diagnostic guidelines-a message of reassurance from an internal medicine doctor in Shanghai"*. **Bischof E**, Chen G, Ferretti MT. Swiss Med Wkly. 2020;150.

· *"ANERGY TO SYNERGY-THE ENERGY FUELING THE RXCOVEA FRAMEWORK"*. **Bischof E**, Broek JA, Cantor CR, Duits AJ, Ferro A, Gao HW, Li Z, de Maria SL, Maria NI, Mishra B. International journal for multiscale computational engineering. 2020;18(3).

· *"Off the back burner: diverse and gender-inclusive decision-making for COVID-19 response and recovery"*. Bali S, Dhatt R, Lal A, Jama A, **Bischof E**, Van Daalen K, Sridhar D. BMJ Global Health. 2020;5(5):e002595.

· *"Efficacy of active rapid molecular screening and IPC interventions to reduce carbapenem-resistant Enterobacteriaceae infections in emergency intensive care unit–a prospective, multi-stage study"*. Wu W, Yang S, He L, Li K, Yu X, Ni L, Hu L, Guo J, **Biskup E**, Tang L. 2019.

· *"Predicting major adverse events in patients with acute myocardial infarction"*. Nestelberger T, Boeddinghaus J, Wussler D, Twerenbold R, **Biskup E**, Badertscher P, Wildi K, Miró s, López B, Martin-Sanchez FJ, Muzyk P. Journal of the American College of Cardiology. 2019;74(7):842-54.

· *"In vivo and in situ real-time fluorescence imaging of peripheral nerves in the NIR-II window"*. Feng Z, Yang Y, Zhang J, Wang K, Li Y, Xu H, Wang Z, **Biskup E**, Dong S, Yang X. Nano Research. 2019;12:3059-68.

· *"Application of circulation tumor cells detection in diagnosis and treatment of breast tumors"*. Cai F, Cen C, Cai L, Falar Luis MA, **Biskup E**. Rejuvenation Research. 2019;22(6):498-502.

· *"The Great Wall: the symbol of a path to great progress in oncofertility services"*. **Biskup E**. Fertility and Sterility. 2019;1(2):1.

· *"Anesthesia with ciprofol in cardiac surgery with cardiopulmonary bypass: A case report"*. Yu L, **Bischof E**, Lu H-H. World Journal of Clinical Cases. 2023;11(1):157.

Curriculum Vitae

Evelyne Bischof (prev. Ewelina Biskup), MD MPH

Specialist in internal medicine with research focus on oncology, longevity and precision medicine. Featured in Forbes magazine, passionate about next-generation medical technology, and the applications of AI for biomedical research and practice, digital health and innovative technology.
Spent a decade practicing medicine and performing translational research in Switzerland, USA, and China. A Harvard- and Columbia-trained physician, author of over 70 peer-reviewed papers e.g. Lancet Oncology, Nature Biotechnology, JAMA Internal medicine, PNAS and JCI.

Frequent speaker at scientific and medical conferences, with a demonstrated history of working in the medical practice industry on international and cross-cultural level.

PERSONAL INFORMATION

Shanghai: cell: +86 150 0086 4674
Email: bischofevelyne@gmail.com
Google scholar
LinkedIn

CURRENT POSITION/FIELD

Professorships
Prof. Shanghai University of Medicine and Health Sciences
Visiting professor Tel Aviv University, School of Medicine
Prof. Jiaotong University, School of Medicine, Shanghai

Internal medicine specialist, chief physician
Renji Hospital, Medical Oncology Department, Jiaotong University School of Medicine, University Hospital of Basel (prev.)

Scientific associate
Clinical Trial Unit, Renji hospital, Shanghai
University of Tel Aviv

EDUCATION AND PROFESSIONAL QUALIFICATIONS

June 2015 **Board certification in Internal Medicine (FMH)**

April 2011 **Faculty of Medicine, University of Dresden**
MD Degree Magna Cum Laude
Max Planck Institute of Molecular Biology and Genetics

POSTGRADUATE CAREER

Employment

November 2022 – now Chief associate physician internal medicine and oncology, Renji Hospital, Jiaotong University School of Medicine, Shanghai

April 2019 – September 2022 Attending physician, internal medicine and oncology, Renji Hospital, Jiaotong University School of Medicine, Shanghai

April 2018 – April 2020 Scientific liaison physician at the Fudan Cancer Institute Shanghai at Fudan University

November 2016 - April 2018 Attending physician, Department of Internal Medicine, University Hospital of Basel

Academic affiliations:

Mai 2020 – present Full Professor Shanghai University of Medicine and Health Sciences

August 2016 – Mai 2020 Associate Professor Shanghai University of Medicine and Health Sciences

March 2018 – present Associate Lecturer Shanghai Jiaotong University

April 2012 - July 2016 Foreign visiting lecturer, Tongji University School of Medicine, Shanghai

Medical residency/Fellowship

Nov 2012 - Nov 2016 Internal Medicine, with rotations in Medical Oncology, Hematology, Emergency Medicine, Intensive Care Medicine Unit, **University Hospital Basel Switzerland**

July 2014 - Nov 2015 SICU/Anesthesia and Internal Medicine Department, University Hospital of Tongji University (Yangpu Central Hospital), Shanghai

Oct 2011 - Nov 2012 **University Hospital Zurich, Switzerland**

Medical internships

April 2010 - Oct 2011 **Columbia University**, **New York**, Presbyterian Hospital & Irving Institute for Clinical and Translational Research, USA (Prof. Harold Pincus)

Nov 2009 - April 2010 **Harvard University Medical School** hospitals

Massachusetts General Hospital,
Dana Farber, Brigham and Women's, Beth Israel Medical Deaconess
July - Nov 2009 General Surgery, Shanghai East Hospital of Tongji University, **Shanghai**, China
August - Sept 2008 **Harvard Medical School**, Radiation Oncology, Cambridge Health Alliance, Boston

Grants

Total amount: 574 000 euro (4 467 322 RMB)

National Grants (258 000 euro, 2 014 981 RMB)

1. SSSTC Sino-Swiss Science and Technology Grant Nov.2015-Nov.2016 "Comparative analysis of colorectal cancer immune contexture and microbial colonization in Swiss and Chinese cohorts of patients: association with clinical pathological characteristics *13 000 euro*
2. Swiss national Foundations (SNF) grant, yearly scientific exchange support grant 2018 Geneva *10 000 euro*
3. Swiss national Foundations (SNF) grant, yearly scientific exchange support grant 2019 Zurich *10 000 euro*
4. German National Academic Stipend **DAAD** for Harvard Medical School medical fellowship, *10 000 euro*
5. German National Merit Foundation (Studienstiftung des Deutschen Volkes) 2010, ***200 000 euro***
6. German National Mittelosteuropa-Stipendium, *10 000 euro*
7. Polish National Stipend of Silesia "ZDolnySlazak" 2004 *5 000 euro*

Scientific Institution grants (314 000 euro, 2 452 341 RMB)

1. Swiss Cancer League Grant 2018-2020 "Oldest Old Cancer Patients" a 2-years scientific grant ***240 000 euro***
2. Rare Disease Grant of the FDIME (Federation for Development of Internal Medicine "PGC1 in male breast cancer – its prognostic and predictive value *10 000 euro*
3. Oncology&Women's Health division Roche grant "Perjeta in advanced HER2+ breast cancer: A comprehensive analysis of pertuzumab use-beyond its label in Switzerland" *18 000 euro*
4. Jiaotong University, Overseas Lecturers Scientific grant, 2018-2019 *24 000 euro*
5. Theodor-Heuss-Stiftung, *10 000 euro*
6. Cusanuswerk *12 000 euro*

Scientific prizes and awards

1. Novartis-poster Price 2015: «NEDD4 promotes cell growth and migration through PTEN/PI3K/AKT signaling pathway in hepatocellular carcinoma» *2 000 euro*
2. Best Poster Award at the **14th European Congress of Internal Medicine,** Moscow, Russia 2015

3. International Women Society of Shanghai, healthcare contributor of the year 2020

LANGUAGES

1. German – native
2. English - fluent written and spoken
3. French – fluent written and spoken
4. Polish – fluent written and spoken
5. Ukrainian – fluent
6. Russian – fluent
7. Italian – basic
8. Mandarin - basic

Memberships in panels, boards, and individual scientific reviewing activities

- **XPrize community** member on Age Reversal
- **RxCoVea consortium framework,** New York University
- **Research Gate** community expert contributor (COVID-19)
- **European Federation of Internal Medicine:** Board Member of the EFIM Young Internists since 2013, Official Delegate of Switzerland
- **Foundation of Internal medicine (FDIME):** Board Member of the Research Committee of the Clinical Research Seminar Paris since 2015
- **Swiss Society of Internal Medicine**: official delegate to the Assembly
- **Swiss Young Internists**, founder and co-president
- **MY AIM** Swiss platform for Internal Medicine board certification: editorial board

Active memberships in further scientific societies

- **Oncofertility Consortium**
  Position: representative of the Oncofertility Program at the Renji Hospital Shanghai
- **Women's Brain Project – Society for Women in Science**
  Position: Executive board member
- **Internal Medicine Assessment of Gender Differences in Europe (IMAGINE), under European**
  Federation of Internal Medicine **(EFIM)**
  Position: Chair

Organization of conferences (selection)

- Member of the scientific committee in congresses, European School of Internal Medicine and Clinical Research Seminar 2016-dato
- Organizing Committee of the **European Congress of Internal Medicine** ECIM 2014-dato
- Organizing Committee of the **Swiss Congress of Internal Medicine** 2014-dato
- Organizing Committee of the **Swiss Great Update** 2014-2016
- Faculty member of the **European School of Internal Medicine**: August 2015 (http://www.esim2015summer.org), June 2016 (http://www.esim2016summer.org),

September 2016 (http://www.esim2016.org), January 2017 (http://www.esim2017.org)

Speaker in telemedia and governmental events (selection)
New England Journal of Medicine podcast
https://catalyst.nejm.org/doi/full/10.1056/CAT.20.0106

Chinese national TV

https://s-url.cgtn.com/p/IaGHcA

https://news.cgtn.com/news/2020-11-07/In-Shanghai-China-meets-the-U-S-innovation-matters-VdxRO3Yk0w/index.html

selected webinars:
https://www.youtube.com/watch?v=97E-4venUIc&feature=youtu.be&app=desktop#menu
https://covid19-1.sciforum.net/
https://www.youtube.com/watch?v=aHNT8PjAaVY
https://www.youtube.com/watch?v=ZndCkoDvalM
https://www.youtube.com/watch?v=SuM5uP2sP6g&t=572s

CCTV "Voices" Shanghai

Publications and Editorial activity
Google scholar:
https://scholar.google.com/citations?user=h1sAumMAAAAJ&hl=en

Editor of "Environmental Research and Public health", special issue on "COVID-19 and SARS-CoV2"
Editorial board member "Praxis – Revue Suisse de la Medicine"
Editorial board member "Precision Cancer Medicine"
Ad hoc reviewer for multiple journals

Speaker at scientific conferences (selection)

USA:	China:	Europe:
San Antonio Breast Cancer Symposium, USA, 2019 "Oncofertility practice in university setting in Shanghai."	***Hangzhou* Xianghu International *Breast Cancer Summit* (HXIBCS)** "Geroncology today" Zhongshan Hospital Fudan University Shanghai Breast Cancer Summit 2016 "Precision medicine in	**DayOne Precision Medicine Innovation Hub, Basel 2019** Speaker: "AI in medicine in China" **European Congress of Internal Medicine (ECIM) Congress, Lisbon 2019** Keynote lecture: "Burn out – the alternative view on the global burden disease" **European Congress of Internal Medicine**

San Antonio Breast Cancer Symposium, USA, 2019 "PGC1alpha plasma level as new prognostic and predictive marker in male breast cancer."	breast cancer" Yangpu Hospital Tongji University Shanghai Breast Cancer Summit 2019 Keynote lecture "Male breast cancer – epidemiology, treatment and challenges" Yangpu Hospital Tongji University Shanghai Breast Cancer Summit 2018 Keynote lecture "Oldest Old Cancer patients" Yangpu Hospital Tongji University Shanghai Breast Cancer Summit 2017 Keynote lecture "Advances in local and systemic breast cancer therapies in Europe" Yangpu Hospital Tongji University Shanghai Breast Cancer Summit 2016 Keynote lecture "Advances in local and systemic breast cancer therapies in Europe" Zhongshan Hospital Fudan University Shanghai Breast Cancer Summit 2018 Keynote lecture "Oldest Old Cancer patients" Zhongshan Hospital Fudan University Shanghai Breast Cancer Summit 2017 Keynote lecture "Advances in breast cancer therapies" Zhongshan Hospital Fudan University Shanghai Breast	**(ECIM) Congress, Wiesbaden 2018** Keynote lecture: "Reflective practice for physicians" **European Congress of Internal Medicine (ECIM) Congress, Milan 2017** Keynote lecture: "Internal medicine doctors on surgical wards" **Swiss Congress of Internal Medicine 2019** 3 keynote lectures: "Migration medicine" "Bone health in oncological patients" "Career as an internist: clinic, teaching and research " **Swiss Congress of Internal Medicine 2018** Keynote lecture: "Medicine, teaching and research in China" **Swiss Congress of Internal Medicine 2017** Keynote lecture: "Medicine, teaching and research as internist" **Swiss Congress of Internal Medicine 2016** Keynote lecture: "The future generation of hospitalists - Swiss Young Internists" **European School of Internal Medicine, Sardinia (summer edition) 2016** Lecture and workshop lead on: "Migration medicine" **European School of Internal Medicine, Sardinia (summer edition) 2015** Lecture and workshop lead on: "Errors in medicine" **European School of Internal Medicine, Riga (winter edition) 2017** Lectures and workshop lead on: "International internist **European School of Internal Medicine, Riga (winter edition) 2016** Lectures and workshop lead on:

	Cancer Summit 2016 Keynote lecture "Personalized therapies in breast cancer" Hangzhou Breast Cancer Summit, 2019 Panel discussion: "Endocrine therapies in breast cancer" Hangzhou Breast Cancer Summit, 2018 Keynote lecture "Oldest Old Cancer patients" Hangzhou Breast Cancer Summit, 2017 Keynote lecture "Advances in breast cancer therapies"	"Migration medicine" "Errors in medicine" **Clinical Research Seminar of the European Federation of Internal Medicine, Paris, 2019** Lectures and workshop lead on: "Rare diseases – male breast cancer" "Research career as internist" **Clinical Research Seminar of the European Federation of Internal Medicine, Paris, 2018** Lecture and workshop lead on: "Research career as internist" **Clinical Research Seminar of the European Federation of Internal Medicine, Paris, 2017** Lecture and workshop lead on: "Research career as internist"

Acknowledgments

I would like to express my heartfelt gratitude and appreciation to all the mentors and individuals who have played a significant role in my journey throughout my PhD and the completion of this thesis. Firstly, I extend my deepest thanks to my PhD directors, Prof. Emanuele Barbato and Prof. Raffaele Izzo, for their invaluable guidance, unwavering support, and continuous encouragement. I am immensely grateful to the entire faculty of the Federico II University, Department of Cardiology, for providing an enriching academic environment and fostering my growth as a researcher.

I would also like to express my profound appreciation to my parents for their unwavering love, encouragement, and belief in my abilities. Their constant support and sacrifices have been instrumental in my academic achievements. Furthermore, I am grateful to my friends for their unwavering friendship and understanding throughout this challenging journey.

My heartfelt thanks go to all my co-authors of the research papers and book chapters for their valuable contributions and collaborative efforts. Each of you has played a pivotal role in shaping and refining the outcomes of this work. I would also like to acknowledge the guidance and mentorship provided by esteemed experts in the fields of cardiology, oncology, AI, and longevity medicine. Special thanks to Prof. Alex Zhavoronkov for his insightful advice and expertise.

I would like to express my gratitude to inspiring specialists in the field, including Prof. Christian Mueller, Prof. Marcus Vetter, Prof. Steven Lindheim, Prof. Michael Wang, Prof. Michael Levitt, Shoshan Levitt, Prof. Nir Barzilai, Prof. Wang Liwei, Prof. Alberto Marra, and many others whose guidance and expertise have shaped my research and broadened my perspective. I regret that I am unable to mention all individuals by name due to the limitations of this thesis.

I would like to thank Yael Maxwell for the picture on the front page from her article: https://www.tctmd.com/news/ai-cardiology-where-we-are-now-and-where-go-next.

Finally, I am grateful to everyone who supported my research endeavors. Your assistance has been instrumental in the successful completion of this thesis.

Once again, my deepest appreciation and thanks to all who have contributed to my academic and personal growth throughout this journey. Your guidance, support, and mentorship have been invaluable, and I am truly honored to have had the opportunity to work with each of you.

„Ein jeder sieht, was er im Herzen trägt“

“A man sees in the world what he carries in his heart.”

Johann Wolfgang von Goethe, Faust