Cumulative Dissertation

Interplay of Influenza and SARS-CoV-2 Infections with Diabetes Mellitus and associated Comorbidities

submitted by

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STATUARY DECLARATION

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by names all those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications, I followed the "Guidelines of the Medical University of Graz on Good Scientific Practice".

Graz, August 2022 e.h. Faisal Aziz

DISCLOSURES

This cumulative dissertation is based on six published articles related to influenza and COVID-19. These articles are presented and duly cited throughout this dissertation as per recommended guidelines of the Medical University of Graz.

Three articles have been published as original articles, with Faisal Aziz as an exclusive first author, at the following SCI-listed journals:

1. **Aziz F**, Aberer F, Moser O, Sourij C, von Lewinski D, Kaser S, Reichardt B, Sourij H. **Impact of comorbidities on mortality in hospitalized influenza patients with diabetes – Analysis of the Austrian Health Insurance.** *Diabetes Research and Clinical Practice.* 2021;174:108758. doi: 10.1016/j.diabres.2021.108758.
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2. **Aziz F**, Aberer F, Bräuer A, Ciardi C, Clodi M, Fasching P, Karolyi M, Kautzky-Willer A, Klammer C, Malle O, Pawelka E, Pieber T, Peric S, Ress C, Schranz M, Sourij C, Stechemesser L, Stingl H, Stöcher H, Stulnig T, Tripolt N, Wagner M, Wolf P, Zitterl A, Reisinger AC, Siller-Matula J, Hummer M, Moser O, von-Lewinski D, Eller P, Kaser S, Sourij H, on behalf of the COVID-19 in Diabetes in Austria Study Group. **COVID-19 In-Hospital Mortality in People with Diabetes Is Driven by Comorbidities and Age-Propensity Score-Matched Analysis of Austrian National Public Health Institute Data.** *Viruses*. 2021;30;13(12):2401. doi: 10.3390/v13122401.
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3. **Aziz F**, Reisinger AC, Aberer F, Sourij C, Tripolt N, Siller-Matula JM, von-Lewinski D, Eller P, Kaser S, Sourij H, on behalf of the COVID-19 in Diabetes in Austria Study Group. **Simplified Acute Physiology Score 3 Performance in Austrian COVID-19 Patients Admitted to Intensive Care Units with and without Diabetes.** *Viruses*. 2022;14(4):777. doi: 10.3390/v14040777.
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5. Sourij C[#], Tripolt NJ[#], **Aziz F[#]**, Aberer F, Forstner P, Obermayer AM, Kojzar H, Kleinhappl B, Pferschy PN, Mader JK, Cvirn G, Goswami N, Wachsmuth N, Eckstein ML, Müller A, Abbas F, Lenz J, Steinberger M, Knoll L, Krause R, Stradner M, Schlenke P, Sareban N, Prietl B, Kaser S, Moser O, Steinmetz I, Sourij H. **Humoral immune response to COVID-19 vaccination in diabetes is age-dependent but independent of type of diabetes and glycaemic control: The prospective COVAC-DM cohort study**. *Diabetes, Obesity and Metabolism*. 2022;24(5):849–58. doi: 10.1111/dom.14643.
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6. Aberer F[#], Moser O[#], **Aziz F[#]**, Sourij C, Ziko H, Lenz J, Abbas F, Obermayer AM, Kojzar H, Pferschy PN, Müller A, Unteregger C, Leitner M, Banfic T, Eckstein ML, Wachsmuth N, Kaser S, Mader JK, Tripolt NJ, Sourij H, for the COVAC-DM Study Group. **Impact of COVID-19 Vaccination on Glycemia in Individuals with Type 1 and Type 2 Diabetes: Substudy of the COVAC-DM Study**. *Diabetes Care*. 2022;45(2):e24–6. doi: 10.2337/dc21-1563.
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FOREWORD

I started my PhD studies a few months before the SARS-CoV-2 outbreak, which not only changed the research focus of my hosting institution but also my research program and dissertation topic. At the beginning of the COVID-19 pandemic, the impact of respiratory viral infections in people with diabetes was not well studied – therefore, we explored this phenomenon using available data in people with influenza in the Austrian Health Insurance database since prospective data on COVID-19 and outcome in people with diabetes was not available then, in particular in Austria. Later in the pandemic, access to the countrywide data on COVID-19 from the GÖG (Gesundheit Österreich GesmbH) platform provided us an evolving picture regarding adverse outcomes of COVID-19 infection in people with diabetes. Although people with diabetes were considered a high-risk population for COVID-19 in the beginning of pandemic, the role of comorbidities was found to be more significant in our analysis of GÖG data. Next, we explored how well the mortality risk in people with diabetes admitted to critical care units could be predicted with a widely utilized prognostication tool. Later, we setup a longitudinal study of COVID-19 patients with diabetes (COVID-19 in diabetes registry) and investigated the role of various biomarkers for predicting the prognosis in this population. Finally, with the availability of COVID-19 vaccine; we prospectively investigated immune and glycemic responses to vaccination in people with diabetes.

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I am thankful to my PhD program “Advanced Medical Biomarker Research” for its support and organizing interesting lectures and seminars for enhancing my knowledge and research skills.

I wish to thank each co-author of my publications for their feedback and suggestions during the process of manuscript publications, which have helped me polish my research manuscripts and this dissertation.

I express my sincerest gratitude to the “*Austrian Health Insurance*”, the “*COVID-19 in Diabetes in Austria Study Group*”, the “*Austrian National Public Health Institute* (GÖG)”, and the “*COVID-19 Vaccination in People with Diabetes Mellitus (COVAC-DM) study”* for granting me access to their datasets that I have used in my publications and this dissertation. Without their cooperation and support, I could not have accomplished my dissertation.

My special gratitude goes to all study participants who provided information to the respective databases for research purposes. This dissertation was possible only because of the invaluable information they provided.

Finally and most importantly, I owe a great debt of gratitude to my beloved family for their endless love, support, and encouragement, and for their understanding throughout the years of my Ph.D. journey.

Table of Contents

LIST OF ABBREVIATIONS

ACCI	Age-adjusted Charlson Comorbidity Index
AHI	Austrian health insurance
AHR	Adjusted hazard ratio
AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted odds ratio
ATC	Anatomical therapeutic classification
AUC, AUROC	Area under the receiver operating characteristics curve
BMI	Body mass index
CABG	Coronary artery bypass grafting
CE	Central Europe
CGM	Continuous glucose monitoring
CHF	Congestive heart failure
CI	Confidence interval
CITL	Calibration in-the-large
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
COVAC-DM	COVID-19 Vaccination in People with Diabetes Mellitus
COVID-19	Coronavirus disease-2019
CSII	Continuous subcutaneous insulin infusion
C-statistic	Concordance statistics
CVD	Cardiovascular disease
DPP4	Dipeptidyl peptidase-4
eGFR	Estimated glomerular filtration rate
FiO_2	Fraction of inspired oxygen
GCS	Glasgow Coma Scale
GÖG	Gesundheit Österreich GmbH (Austrian National Public Health Institute)
GLP-1RA	Glucagon-like peptide-1 receptor agonist
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
H-L	Hosmer-Lemeshow
HR	Hazard ratio
ICD	International classification of disease

ICH GCP	International conference on harmonization for good clinical practice
ICU	Intensive care unit
IRI	Influenza related illness
IQR	Interquartile range
LDL	Low density lipoprotein
MDI	Multiple daily insulin injections
mRNA	Messenger ribonucleic acid
NYHA	New York heart association
ÖDG	Österreichische Diabetes Gesellschaft
OR	Odds ratio
OHA	Oral hypoglycemic agents
PAD	Peripheral artery disease
PaO_2	Partial pressure of oxygen in arterial blood
PTCA	Percutaneous transluminal coronary angiography
PSM	Propensity score matching
PVD	Peripheral vascular disease
r	Pearson correlation coefficient
RBD	Receptor-binding domain
SAPS 3	Simplified Acute Physiology Score 3
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SGLT2	Sodium glucose co-transporter-2
SMD	Standardized mean difference
SMR	Standardized mortality ratio
STROBE	Strengthening the Reporting of Observational studies in Epidemiology
SD	Standard deviation
TAR	Time above range
TBR	Time below range
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TIR	Time in range
USA	United States of America
WHO	World Health Organization

ZUSAMMENFASSUNG

Ziele

Diabetes gilt als einer der Hauptrisikofaktoren für Influenza- und SARS-CoV-2-Infektionen und deren nachteilige Folgen. Die Gründe, die Diabetes zu einer Hochrisikokrankheit machen, sind jedoch unklar. Auch gibt es nur wenige Belege für die Leistungsfähigkeit von Risikoscores und Biomarkern zur Vorhersage der Prognose von schwerkranken COVID-19-Patienten mit Diabetes. Darüber hinaus müssen die immunogene Reaktion auf die COVID-19-Impfung und ihre Auswirkungen auf die vorübergehende Hyperglykämie bei Menschen mit Diabetes noch untersucht werden.

Methoden

Die Datenbank der österreichischen Krankenversicherung (AHI) (N = 504.184) wurde analysiert, um den Einfluss klinischer Merkmale, Komorbiditäten und des ACCI auf die Hospitalisierungs- und Kurzzeitsterblichkeitsrate nach einer grippebedingten Infektion (IRI) zu untersuchen. Die GÖG-Datenbank (N = 40.602) wurde analysiert, um die Auswirkung von Diabetes auf die Aufnahme in die Intensivstation (ICU) und die Krankenhausmortalität nach COVID-19 mithilfe der Propensity-Score-Matching-Methode zu untersuchen. Das Register COVID-19 bei Diabetes (N = 747) untersuchte die prognostische Wertigkeit verschiedener Biomarker zur Vorhersage der Krankenhausmortalität. Die COVAC-DM-Studie (N = 160) untersuchte den Einfluss von Typ-1- und Typ-2-Diabetes und der glykämischen Kontrolle auf die Antikörperreaktion nach der ersten und zweiten COVID-19-Impfung.

Ergebnisse

In der AHI-Datenbank waren Personen mit hohem Alter, Komorbiditäten und einem höheren ACCI häufiger wegen einer IRI hospitalisiert als Personen, die aus anderen Gründen hospitalisiert wurden. Die bereinigte Hazard Ratio (aHR) für die 90-Tage-Mortalität stieg mit dem Alter (aHR: 3,00 bis 7,15 für 50-59 bis 80+ Jahre), der Herzinsuffizienz (aHR: 1,97, 95%-Konfidenzintervall [CI]: 1,31-2,98), der Nierenerkrankung (aHR: 1,50, 95%CI: 1,05-2,14) und dem ACCI (aHR: 1,14, 95%CI: 1,08-1,19). In der GÖG-Datenbank war Diabetes nach dem Propensity Matching nicht signifikant mit der In-Hospital-Mortalität assoziiert (Odds Ratio [OR]: 1,08, 95%CI: 0,97-1,19), während er signifikant mit der Aufnahme auf die Intensivstation verbunden war (OR: 1,15, 95%CI: 1,04-1,28). Bei den GÖG-Intensivpatienten lag die SAPS-3-Diskriminierung bei 69 % und war bei Patienten mit und ohne Diabetes ähnlich (70% vs. 68%, p = 0,193). Der Brier-Score lag bei >0,20 und die Kalibrierung war für alle SAPS-3-

Versionen bei allen Patienten unzureichend (jeweils p <0,05). Im COVID-19-Register waren LDH, CRP, IL-6, PCT, AST-ALT-Verhältnis, NT-proBNP und Troponin T signifikant mit der In-Hospital-Mortalität assoziiert. Von allen Biomarkern zeigte NT-proBNP eine gute Diskriminierung (74%) und Kalibrierung (p = 0,302), während Troponin T eine gute Diskriminierung (81%), aber eine schlechte Kalibrierung (p = 0,010) zeigte. In der COVAC-DM-Studie waren die Antikörperspiegel zwischen Typ-1-Diabetes, Typ-2-Diabetes, gut und unzureichend eingestellter Glukose und gesunden Kontrollen ähnlich (p >0,05). Die Antikörperreaktion nahm mit zunehmendem Alter ab (r = -0,45, p <0,001) und stieg mit zunehmender eGFR (r = 0,28, p = 0,001). In der COVAC-DM-Teilstudie unterschieden sich die Zeit im Bereich (p = 0,962, p = 0,704), die Zeit unter dem Bereich (p = 0,952, p = 0,704) und die Zeit über dem Bereich (p = 0,941, p = 0,715) für Glukose bei Teilnehmern mit Typ-1- und Typ-2-Diabetes nicht signifikant um den Impfzeitraum. Teilnehmer mit Typ-1-Diabetes verbrachten an Tagen mit einem Nebenwirkungs-Score >0 im Durchschnitt weniger Zeit im Bereich (p = 0,045) und mehr Zeit über dem Bereich (p = 0,040). Bei Teilnehmern mit Typ-2-Diabetes veränderte der Nebenwirkungs-Score den Blutzuckerspiegel nicht. Die COVID-19-Impfung hatte keinen Einfluss auf die Insulindosierung (p = 0,578, p = 0,346) und die Kohlenhydratzufuhr (p = 0,092, p = 0,958) bei Teilnehmern mit Typ-1- und Typ-2-Diabetes.

Konklusion

Alter, Herzinsuffizienz, Nierenerkrankung und ACCI sagten die Kurzzeitmortalität bei hospitalisierten IRI-Patienten mit Diabetes signifikant voraus. Menschen mit Diabetes hatten ein höheres Risiko, eine schwere COVID-19 zu entwickeln, nicht aber die Mortalität, und die beobachtete Mortalität wurde durch Alter und Multimorbidität beeinflusst. Die unzureichende Leistung des SAPS 3 bei Diabetes- und Nicht-Diabetes-Patienten deutet darauf hin, dass er sich nicht als Prognoseinstrument für COVID-19-Patienten eignet. NT-proBNP und Troponin T zeigten bei COVID-19-Patienten mit Diabetes eine gute Vorhersagekraft. Die Antikörperreaktion nach der zweiten COVID-19-Impfung war bei Patienten mit und ohne Diabetes sowie bei Patienten mit gut und schlecht eingestelltem Blutzuckerspiegel ähnlich. Die COVID-19-Impfung veränderte den Blutzuckerspiegel bei Menschen mit Diabetes nicht signifikant. Bei Teilnehmern mit Typ-1-Diabetes traten jedoch an Tagen mit Nebenwirkungen signifikante Veränderungen des Blutzuckerspiegels auf.

Schlüsselwörter: Biomarker; COVID-19; Komorbidität; Diabetes; Glykämie; Influenza; Intensivstation; Sterblichkeit; Beobachtungsstudie; SARS-CoV-2; Simplified acute physiology score; SAPS 3; Typ 1 Diabetes; Typ 2 Diabetes; Impfung

ABSTRACT

Purpose

Diabetes is considered a major risk factor for influenza and SARS-CoV-2 infections and their adverse outcomes. However, the underlying reasons that make diabetes a high-risk condition are unclear. Also, evidence on the performance of risk scores and biomarkers to predict the prognosis of seriously ill COVID-19 patients with diabetes is scarce. Moreover, the immunogenic response to COVID-19 vaccination and its impact on transient hyperglycaemia are yet to be investigated in people with diabetes.

Methods

The Austrian Health insurance (AHI) database (N = 504,184) was analyzed to investigate the impact of clinical characteristics, comorbidities, and age-adjusted Charlson Comorbidity Index (ACCI) on hospitalization and short-term mortality rates following influenza-related infection (IRI). The GÖG database (N = 40,602) was analyzed to investigate the impact of diabetes on intensive care unit (ICU) admission and in-hospital mortality following COVID-19 using the propensity score matching method. In-hospital mortality among ICU patients with COVID-19 (N = 5850) was predicted using global, Central European, and Austrian versions of SAPS 3. The COVID-19 in diabetes registry (N = 747) investigated the performance of various haematological, coagulation, inflammatory, hepatic, and cardiac biomarkers to predict in-hospital mortality. The COVAC-DM study (N = 160) investigated the impact of type 1 and type 2 diabetes and glycaemic control on antibody response after the first and second COVID-19 vaccinations. Also, short-term effects (2 days before and 3 days after the vaccination) of COVID-19 vaccination on glycaemia, insulin dosage, and carbohydrate intake were investigated in COVAC-DM participants on a continuous glucose monitoring system and insulin therapy (N = 74).

Results

In the AHI database, people with old age, comorbidities, and a higher ACCI were more likely to be hospitalized for IRI than those hospitalized for other reasons. The adjusted hazard ratio (aHR) for 90-day mortality increased with age (aHR: 3.00 to 7.15 for 50–59 to 80+ years), heart failure (aHR: 1.97, 95% confidence interval [CI]: 1.31–2.98), renal disease (aHR: 1.50, 95%CI: 1.05–2.14), and ACCI (aHR: 1.14, 95%CI: 1.08–1.19). In the GÖG database, diabetes was not significantly associated with in-hospital mortality (odds ratio [OR]: 1.08, 95%CI: 0.97–1.19) after propensity matching, while it was significantly associated with ICU admission (OR:

1.15, 95%CI: 1.04–1.28). In GÖG ICU patients, the SAPS 3 discrimination was 69% and was similar between diabetes and non-diabetes patients (70% vs. 68%, p = 0.193). The Brier score was >0.20 and calibration was inadequate (p <0.05 each) for all SAPS 3 versions in the entire, diabetes, and non-diabetes patients. In the COVID-19 registry, LDH, CRP, IL-6, PCT, AST-ALT ratio, NT-proBNP, and Troponin T were significantly associated with in-hospital mortality. Of all biomarkers, NT-proBNP showed good discrimination (74%) and calibration (p = 0.302), while Troponin T showed good discrimination (81%) but poor calibration (p = 0.010). In the COVAC-DM study, antibody levels were similar (p >0.05) between type 1 diabetes, type 2 diabetes, well and insufficiently controlled glucose, and healthy controls. The antibody response decreased with increasing age (r = –0.45, p <0.001) and increased with increasing eGFR (r = 0.28, p = 0.001). In the COVAC-DM sub-study, time in range (p = 0.962, p = 0.704), time below range (p = 0.952, p = 0.704), and time above range (p = 0.941, p = 0.715) for glucose did not vary significantly around the vaccination period in type 1 and type 2 diabetes participants. Type 1 diabetes participants spent less average time in range (p = 0.045) and a higher time above range (p = 0.040) on days with a side effect score >0. The side effect score did not alter glycaemia in type 2 diabetes participants. The COVID-19 vaccination did not alter insulin dosage (p = 0.578, p = 0.346) and carbohydrate intake (p = 0.092, p = 0.958) in type 1 and type 2 diabetes participants.

Conclusions

Old age, heart failure, renal disease, and ACCI significantly predicted short-term mortality in hospitalized IRI people with diabetes. People with diabetes were more likely to develop severe COVID-19 but not its mortality, and the observed mortality was influenced by age and multimorbidity. The inadequate performance of SAPS 3 in diabetes and non-diabetes patients suggested that it is not a suitable prognostication tool for COVID-19 patients. NT-proBNP and Troponin T demonstrated good predictive performance in COVID-19 patients with diabetes. The antibody response after the second COVID-19 vaccination was similar in people with and without diabetes and those with well and poorly controlled glycaemia. The COVID-19 vaccination did not significantly alter glycaemia in people with diabetes. However, significant glycaemic changes occurred in type 1 diabetes participants on days with side effects.

Keywords: Biomarker; COVID-19; Comorbidity; Diabetes; Glycaemia; Influenza; Intensive care unit; Mortality; Observational study; SARS-CoV-2; Simplified acute physiology score; SAPS 3; Type 1 diabetes; Type 2 diabetes; Vaccination

1. INTRODUCTION

1.1. Overview of diabetes mellitus

Diabetes mellitus is a chronic metabolic disorder that is characterized by increased blood glucose levels due to either insufficient production of insulin or the inability of the body to effectively use insulin. It is also accompanied by other metabolic abnormalities (1).

1.2. Types of diabetes mellitus

Diabetes is classified into four types as per American Diabetes Association guidelines. Two common types of diabetes include type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is an autoimmune disorder characterised by insulin deficiency due to immunogenic loss of pancreatic β cells. In T1DM, external administration of insulin is required to manage blood glucose and survive. T2DM is characterised by a progressive decline in insulin secretion and the development of insulin resistance. Clinical manifestations of both T1DM and T2DM include polyuria, polydipsia, increased appetite, weight loss, vision changes, and fatigue (1). These symptoms may manifest suddenly in days or weeks in T1DM. While in T2DM, symptoms are less obvious and may appear over months or years (2). Gestational diabetes is developed during the second or third trimester of pregnancy. Other types of diabetes are caused by miscellaneous underlying reasons including monogenic defects in β cells, genetic defects in insulin action, endocrinopathies, exocrine pancreatic pathologies, and other disorders (1). In this dissertation, the emphasis is laid on T1DM and T2DM; therefore, other types of diabetes will not be discussed further.

1.3. Pathophysiology and aetiology of diabetes mellitus

1.3.1. Pathophysiology of type 1 diabetes mellitus

In T1DM, insulin-secreting β cells in the islets of Langerhans in the pancreas are destroyed due to an autoimmune process in β cells. The β cells release autoantigens during cellular turnover or damage, which are recognized and taken up by antigen-presenting cells (APCs) such as macrophages and dendritic cells residing in the islets of the pancreas. Afterward, these APCs produce interleukin (IL)-12 which activates CD4+ T cells, which then

triggers the production of IL-2, followed by the activation of CD8+ T cells specific to β-cell antigens. These activated T cells then differentiate into cytotoxic T cells and are transported into pancreatic islets, where they destroy the β cells. The β cells are also destroyed by granzymes and perforin produced by the CD8+ T cytotoxic cells, cytokines, and reactive oxygen molecules produced by the islet macrophages. Furthermore, insulitis may also mediate the destruction of β cells (3–5).

The pancreatic autoantibodies responsible for destroying β cells include islet cell autoantibodies, antibodies to insulin, glutamic acid decarboxylase, insulinoma-associated autoantigen 2, and protein tyrosine phosphatase (4,6). These antibodies are detectable in ~90% of people with recently diagnosed T1DM and can appear in the serum of susceptible people even years before the onset of T1DM. The risk of T1DM has been shown to increase with the number of circulating autoantibodies. However, this relationship is significantly influenced by various factors such as the age of seroconversion, specific combinations of autoantibodies, and genotypes of autoantibodies. Besides autoantibodies, insulitis, which is characterized by severe infiltration of T cells and macrophages, can also destroy β cells (4–6).

Autoimmune destruction of β cells results in insulin deficiency and eventually hyperglycaemia. The rate of autoimmune destruction of β cells determines the progression of T1DM. For instance, in some cases, the β-cell destruction occurs rapidly, which results in diabetic ketoacidosis (DKA). While in other cases, the destruction of β-cell occurs progressively with a gradual increase in fasting blood glucose levels. In such cases, DKA develops when an individual experiences any acute physiological stressful condition such as severe infection or other disorders. In adult-onset T1DM, β cells retain some functionality to secrete the amount of insulin that is sufficient to prevent ketoacidosis. However, progressive insulin deficiency leads to severe hyperglycemia followed by DKA. Even though T1DM progresses differently, people require extraneous insulin therapy sooner or later to manage their hyperglycaemia. Regardless of insulin deficiency at any age, people with T1DM manifest low or undetectable levels of plasma C-peptide (6,7).

1.3.2. Aetiology of type 1 diabetes mellitus

Various genetic, immunogenic, physiological, and environmental factors are considered responsible for triggering the autoimmune process in T1DM.

The primary genetic factor responsible for producing autoantibodies is the human leukocyte antigen (HLA) class II region of the major histocompatibility complex (MHC).

Specifically, the genes involved in encoding MHC production, antigen processing, and T-cell receptor binding protein contribute to the development of autoantibodies. The HLA-associated genes altogether account for 50–60% of the overall genetic susceptibility to autoimmunity in T1DM. Various other genes within and outside the HLA region can influence aspects of immune responses and increase the vulnerability of β cells to inflammatory markers. A significant role of genetic interaction in triggering autoimmunity has also been recognized. As such, collective expression of multiple HLA genes has demonstrated a stronger association with autoimmunity than the expression of a single gene. Genome-wide association studies have discovered nearly 60 loci to be linked with T1DM, indicating the polygenic nature of this disease (5,8).

Genetic factors contribute to 40% of T1DM only, and a significant rise in the incidence of T1DM in genetically vulnerable children has been witnessed, suggesting the crucial contribution of non-genetic factors in the aetiology of this disease (5). However, the exact contribution of non-genetic factors in T1DM is less understood. These factors, which include viral infections, gut microbiome, diet, and immunological changes, have been shown to exacerbate the autoimmune process in β cells during the early years of life (5).

Viral infections are among the main environmental factors that can cause T1DM. Although exact mechanisms of virus-induced diabetes are unknown, three mechanisms such as bystander activation, epitope spreading, and molecular mimicry have been suggested to induce the autoimmune response in T1DM (4). The viruses associated with T1DM development include enteroviruses, cytomegalovirus, mumps virus, rotavirus, and respiratory viruses. Antibodies against enterovirus and their RNAs have been detected in newly diagnosed T1DM people and pregnant women and their children (4,5). Also, temporal changes in the incidence of T1DM have been observed in association with viral infections, suggesting the significant role of viruses in inducing autoimmunity. Similarly, the incidence of T1DM has been associated with outbreaks of mumps, rubella, and human enterovirus B. Autopsy and biopsy studies have also confirmed the presence of viral infections in pancreatic islets of people with T1DM. Concerning respiratory viruses, the onset of T1DM has been shown to increase in children infected with H3N2, Influenza B, and parainfluenza viruses during the season of viral infections. Meanwhile, the likelihood of developing islet β-cell autoantibodies has been found to increase due to respiratory infections. Likewise, a 20% higher risk of developing T1DM was reported after the H3N1 pandemic (4,5).

Alterations in the gut microbiome have been linked with the induction and progression of the autoimmune process in T1DM. The distribution of microbial groups has been reported to

vary significantly between different autoimmune diseases. In a population with a high incidence of T1DM, more lipopolysaccharides are produced by Bacteroides species. In addition, these lipopolysaccharides are less likely to activate the innate immune response and develop tolerance than the lipopolysaccharides produced by Escherichia coli in a population with a low incidence of T1DM. Likewise, Bacteroides species have been found abundant in children with multiple autoantibodies compared to healthy controls. Moreover, butyrate-producing species were less common compared with Firmicutes in children with autoantibodies. In addition, a large cohort study demonstrated that bacterial pathways related to short fatty acid production were reduced in children with positive islet antibodies or T1DM. Although evidence suggests a significant association between microbiome composition and T1DM, it remains unknown whether these changes are induced by T1DM itself or environmental factors such as infection, diet, agriculture, food product preparation, personal hygiene, and use of antibiotics during the early years of life (5).

The role of diet in T1DM induction is not well-established yet. However, studies have reported a significant association of cow milk consumption with the risk of T1DM because insulin found in this milk triggers the immune response. Moreover, experimental studies have claimed that albumin (ABBOS) present in cow milk has the potential to act as a self-reactive epitope because of its resemblance with the p69 protein located on the surface of β cells. The presence of toxins in cereals and their early introduction into the diet and intake of nitrate with water may also trigger an autoimmune response at an early age. Likewise, a diet low in omega-3 fatty acids and Vitamin D deficiency may elevate the risk of T1DM (5,8).

Various β-cell-related factors, immunological factors, and physiological abnormalities in the bone marrow, thymus, and immune system altogether may significantly contribute to the development of T1DM (4,5). Interestingly, evidence reveals that people diagnosed with T1DM have lower pancreatic, β-cell, and α-cell masses than healthy controls. However, the pancreatic mass was not significantly associated with the duration of T1DM, suggesting that both genetic and environmental factors may contribute to pancreatic atrophy long before the onset of the disease. Various environmental and immunological factors such as immune and physiological disturbances originating in the thymus, bone marrow, and lymphocytes are known to inflict stress on β cells, which leads to protein misfolding or the production of autoantibodies and eventually apoptosis of β cells (4,5,8).

1.3.3. Pathophysiology of type 2 diabetes mellitus

T2DM is characterized by various chronic metabolic derangements associated with hyperglycaemia. Its development and progression are driven mainly by two pathophysiological mechanisms: 1) insulin resistance and 2) β-cell dysfunction. As precise secretion and action of insulin are required for optimal glucose metabolism; hence, disturbances in any of these mechanisms can disrupt glucose regulation and eventually lead to T2DM. In β-cell dysfunction, insulin secretion reduces, which results in hyperglycaemia. Insulin resistance develops due to abnormalities in various cellular pathways, which decreases insulin sensitivity of receptors in the muscle, liver, and fat cells in the peripheral tissues. Due to insulin resistance, glucose production increases in the liver, and its uptake decreases in muscle, liver, and adipose cells. In the early stages of T2DM, decreased insulin sensitivity of peripheral cells triggers β cells to secrete more insulin by increasing their number, size, and function to maintain normal glucose levels. As a result, hyperinsulinemia develops; however, blood glucose concentration remains within its normal range because of the reduced efficacy of insulin signalling. As the disease progresses, the hypersecretion of insulin is unable to compensate for insulin resistance, resulting in the gradual loss of β-cell function and volume. The β-cell dysfunction eventually leads to insulin deficiency and subsequently hyperglycaemia. Obesity plays a fundamental role in developing insulin resistance, as adipose tissue, particularly around the abdomen, precipitates insulin resistance by several inflammatory mechanisms, releasing free fatty acids and dysregulating adipokines (4,9,10).

In T2DM, β-cell dysfunction occurs due to complex interaction between molecular and environmental pathways. Obesity is often accompanied by hyperglycaemia and hyperlipidemia. These metabolic abnormalities promote insulin resistance and chronic inflammation. Also, due to these abnormalities, genetically susceptible β cells may undergo inflammation, endoplasmic reticulum, metabolic/oxidative, and amyloid stress, which increases the risk of compromising islets integrity and ultimately β-cell damage. Obesity-induced production of free fatty acids, hyperglycaemia, lipotoxicity, glucotoxicity, and glucolipotoxicity can also induce endoplasmic reticulum, metabolic, and oxidative stress, thereby resulting in reduced insulin secretion and β-cell apoptosis. Apart from these mechanisms, defects in the synthesis of insulin precursors such as proinsulin, insulin itself, and secretory mechanism can disrupt optimal insulin secretion and its regulation (9,10).

In T2DM, the exact mechanisms of insulin resistance development are poorly understood. Studies suggest that a defect in insulin-stimulated glucose transport in skeletal muscles is responsible for insulin resistance. Obesity-induced excessive production of free

fatty acids has been shown to promote this defect by inhibiting the insulin-stimulated phosphorylation process. Obesity also promotes systemic inflammation, which is characterized by impairment in cytokine production (TNF-α and IL-6) and inflammatory signalling cascades. This obesity-induced inflammatory response can induce insulin resistance by directly influencing the phosphorylation process of glucose transport in hepatic and skeletal cells or disrupting lipid metabolism. Also, disturbances in fatty-acid metabolism resulting from excess energy consumption or defects in fat metabolism in mitochondria lead to excessive accumulation of lipids in muscles, liver, and pancreatic β cells. The excessive accumulation of fatty acids in these cells results in the development of insulin resistance (9,11).

1.3.4. Aetiology of type 2 diabetes mellitus

The aetiology of T2DM is multifactorial, which is driven by a complex interaction among various genetic, metabolic, and environmental factors. Concerning genetic factors, nearly 222 genes have been linked with the development of T2DM. The most significant gene associated with the risk of T2DM is the Transcription factor 7 like 2 gene, which along with obesity, advances the autoimmunity of pancreatic islets. Besides, the human leukocyte antigen gene has been suggested to cause T2DM by intensifying the immune response and thereafter β-cell damage. Genetic studies have discovered more than 100 susceptibility loci in T2DM and have declared it a polygenic disease. In addition, the majority of these genes influence insulin secretion and adipogenesis, while a few genes compromise insulin action (9). Epigenetic factors such as chromosomal DNA methylation, histone modifications, and non-coding RNA regulations have also been found associated with T2DM. Underexpression of insulin encoding, insulin promoter factor-1, peroxisome proliferator-activated receptor-gamma coactivator, and Glucagon-like-peptide receptor genes have increased the DNA methylation in people with T2DM as compared to those without T2DM. Posttranslational modifications in the histone are suggested to modify chromatin structure and induce T2DM. Also, epigenetic changes during the RNA slicing are noted in T2DM patients (4).

Besides the genetic and epigenetic factors, environmental factors such as obesity, high calorie-diet, air pollution, low physical activity, and viral infections can trigger the development and progression of T2DM. The most common viruses that have been found associated with T2DM are cytomegalovirus and respiratory syncytial virus. In a prospective cohort study from Korea, people infected with cytomegalovirus had a higher incidence of T2DM (6% vs. 2%) compared to controls. According to a case study, a person developed T2DM after suffering from the respiratory syncytial virus infection. Another study in old adults showed that people who had cytomegalovirus were twice likely to develop T2DM than controls (4,9).

Obesity has been identified as the most prominent risk factor for T2DM and its associated metabolic derangements. Specifically, obesity increases levels of free fatty acids, glycerol, hormones, cytokines, and proinflammatory markers, thus playing a major role in developing insulin resistance, β-cell dysfunction, and ultimately T2DM. In addition, abdominal fat is more lipolytic than subcutaneous fat and does not respond well to insulin, making it a more significant risk factor for insulin resistance (9,12).

A high-calorie diet is considered another significant risk factor for T2DM. This diet contains a high amount of fats and carbohydrates, hence increasing blood glucose, very low-density lipids, chylomicrons, and triglycerides. These metabolic changes trigger reactive oxygen species (ROS), leading to the production of inflammatory molecules, which altogether elevates the risk of insulin resistance and T2DM (9,10).

Low physical activity or a sedentary lifestyle is another established risk factor for T2DM. Physical activity offers various benefits that can delay the onset and progression of T2DM. It substantially improves glucose uptake by muscles from plasma. It also reduces intra-abdominal fat, which is an established risk factor for insulin resistance. In addition, adequate physical activity has been demonstrated to reduce inflammation and oxidative stress. It also serves as a link between obesity and T2DM. Evidence also suggests that sedentary life and low physical activity promote the development of chronic metabolic inflammation by increasing concentrations of IL-6, IL-1, TNF-α, and C-reactive protein (CRP) in the bloodstream, which are known to trigger an autoimmune response in β cells, inhibit the function of β cells, and promote β-cell apoptosis. On the other hand, adequate physical activity reduces the circulatory levels of inflammatory markers and T2DM-related oxidative stress and improves glucose tolerance (9,10).

Smoking has also been linked with the risk of T2DM via two pathophysiological mechanisms. First, smokers are more likely to be centrally obese than non-smokers, driven mainly by nicotine signalling. Second, nicotine in cigarettes and other tobacco products has been shown to decrease insulin production, increase insulin resistance and compensatory insulin-secretion responses, and impair β-cell function. The literature also reports a strong dose-response relationship between cigarette smoking and the risk of T2DM (10).

According to recent evidence, the gut microbiome may also contribute to the development of diabetes. In this perspective, changes in dysbiosis have been linked with the development of insulin resistance and T2DM. The fat-rich diet has been shown to increase lipopolysaccharide production by threefold in mice, hence contributing to low-grade inflammation and insulin resistance. In addition, gut dysbiosis has been shown to decrease

the synthesis of short-chain fatty acids, which are involved in maintaining gut integrity, the proliferation of β cells, and insulin biosynthesis. Dysbiosis can also compromise the production of branched-chain amino acids and trimethylamine, thus disturbing the homeostasis of glucose and triggering T2DM development (9).

Mitochondrial dysfunction has also been associated with insulin resistance and T2DM development. However, the nature of this relationship is bidirectional. For instance, insulin resistance, a central component of T2DM, can result in mitochondrial dysfunction via nutrient overload, followed by the accumulation of ROS. In contrast, mitochondrial dysfunction caused by oxidative stress, defective biogenesis, genetic mutations, and aging may also lead to the development of T2DM (9).

1.4. Epidemiology of diabetes mellitus

1.4.1. Type 1 diabetes mellitus

T1DM is a less common type of diabetes, representing 5–10% of the total disease. Although T1DM can develop at any age, its onset is more frequent in children and adolescents. It is amongst the leading chronic diseases in children, and its usual age of onset is 4–6 years and 10–14 years, with a peak incidence at 5–7 years (13). Approximately 1.2 million children and adolescents aged 0–19 years are affected by T1DM globally, while ~184,100 are diagnosed with T1DM each year (13). Around 1.9 million children and adults are living with T1DM in the United States, which is expected to increase to 5 million by 2050 (14). The global incidence of T1DM is estimated to be 15 per 100,000 people, and its prevalence is 9.5 per 10,000 people (15). The incidence of child- and adult-onset T1DM is higher in males than in females (16).

The burden of T1DM is rising steadily worldwide, with an annual increase in the incidence of 2–5% (17,17). Meanwhile, the prevalence of T1DM increased by 21% from 2001 to 2009. It is projected that the incidence of T1DM will double in the next decade (16). However, the rate of increase in the incidence is not similar across all age groups. For instance, in Europe, a more pronounced rise has been noted in children under 5 years, while its incidence has stabilized in Sweden. Data show mixed trends in the incidence of adult-onset T1DM. Studies from Finland, Serbia, Sweden, Korea, Taiwan, and the United States have reported a decline in the incidence of adult-onset T1DM from 1983 to 2017. In contrast, studies have reported stable incidence rates in Spain from 2009 to 2016, in the United Kingdom from 1994

to 2013, and in Hong Kong from 2002 to 2015. Whereas data from Mali and Scotland showed an increase in the incidence of T1DM in adults from 2007 to 2016 and 2012 to 2019, respectively (16,18).

The burden of T1DM in children and adolescents varies considerably by geographic region and country. The rates of T1DM among children and adolescents aged 0–19 years are the highest in Northern Europe, followed by Southeast Asia, the Middle East, and North Africa. In addition, Finland and other Northern European countries have approximately 400-fold higher incidence rates of T1DM than China and Venezuela (16,18). A recent meta-analysis has estimated the highest T1DM incidence of 20 per 100,000 population in America, followed by 15 per 100,000 in Asia and Europe each, and the lowest incidence of 8 per 100,000 population in Africa (15). A comparison among countries shows the highest prevalence of T1DM (229,400) in children and adolescents in India, followed by the United States (157,900) and Brazil (92,300) due to their large populations (16,18).

The geographic trends in the incidence of adult-onset T1DM somewhat resemble child-onset T1DM. In adults, the annual incidence of diabetes varies 30-fold among countries. As such, the incidence was 1 per 100,000 in China compared to 30 per 100,000 in Northern Europe and Eastern Africa (18). According to a recent systematic review, the incidence of adult-onset T1DM was the lowest in Asian countries including China, Taiwan, and Hong Kong. While it was the highest in Nordic countries such as Sweden, Finland, Norway, and Denmark. The incidence of T1DM has been shown to correlate with seasonal changes as well. More cases of T1DM are diagnosed in autumn and winter (16).

T1DM contributes to significant mortality worldwide. A recent aggregated analysis from Denmark, Latvia, Scotland and Spain, Australia, and the United States has reported 18,105 deaths per 1.55 million person-years among 179,514 people (0–79 years) with T1DM. More deaths were reported in males (11,355) compared to females (6750), with a ratio of 1.68. The crude all-cause mortality rate for T1DM was 11.7 per 1000 person-years and was higher in males (13.2) than females (9.9). Moreover, the age- and sex-standardized all-cause annual mortality rates declined in all included countries ranging from −2% to −6% during the study period (19).

1.4.2. Type 2 diabetes mellitus

T2DM is the most frequently occurring diabetes, accounting for 90–95% of the total disease (18). It usually develops in middle age and older adults, with a peak incidence at 55-59 years. The international diabetes federation estimates that approximately 541 million adults

are at risk of developing T2DM worldwide. Currently, an estimated 462 million people are living with T2DM, which accounts for 9% of the world's adult population (18). Its global prevalence is estimated to be 6059 cases per 100,000 population. The actual disease burden of T2DM is believed to be underestimated, as approximately every 1 in 3 people are unaware of its existence (20).

T2DM is constantly increasing worldwide due to population ageing, economic development, urbanization, and a concurrent rise in the burden of its established risk factors such as obesity, sedentary lifestyle, and unhealthy diet. The global prevalence of T2DM is predicted to increase to 7079 per 100,000 by 2030 and 7862 by 2040 (20). As per global disease burden data, the prevalence of T2DM has increased by 49% since 1990. The absolute number of people with T2DM increased from 148.6 million in 1990 to 437.9 in 2019. This increase was more pronounced in South Asia, East Asia, and Western Europe (21). Alarmingly, the incidence of T2DM is increasing in early age groups, perhaps due to an increase in the burden of various risk factors in this population (20).

The burden of T2DM varies considerably among geographic regions. Pacific Ocean Island countries such as Fiji (20,277), Mauritius (18,545), American Samoa (18,312), and Kiribati (17,432) have the highest prevalence (per 100,000 population) of T2DM (20). While its prevalence has increased in Southeast Asian countries in the last two decades. Compared to South Asia, Western Europe has a higher prevalence of T2DM, which continues to rise progressively despite drastic public health measures. China, India, and the United States of America have the highest number of people with T2DM, respectively, due to their large populations. The prevalence of T2DM is slightly higher in males (6219 per 1000,000) compared to females (5898 per 100,000). Also, it seems to develop at an early age in males than females. Racial disparity in T2DM has also been observed. As such, it occurs more frequently in Native Americans, Asian Americans, African Americans, Hispanic, and Latinos, suggesting the genetic origin of this disease (20).

T2DM is the 9th leading cause of death worldwide. In 2017, it accounted for 1 million deaths globally. It is the 7th leading disease with respect to disability-adjusted life years (DALY) (18,20). According to the global disease burden data, T2DM associated mortality rate is increasing over time. For instance, T2DM led to 1472.9 thousand deaths in 2019, and its global age-standardized death rate was 18.5 per 100,000 population, which was 10.8% higher than its rate in 1990. Likewise, the global age-standardized DALY rate was 801.5 per 100,000 population in 2019, which was 27.6% higher than in 1990. With respect to absolute numbers,

deaths related to T2DM increased from 606.4 thousand in 1990 to 1472.9 thousand in 2019. Most deaths were observed in South Asian, Southeast Asian, and East Asian countries (21).

1.5. Diagnosis of diabetes mellitus

T1DM and T2DM are diagnosed by glycated haemoglobin (HbA1c), fasting blood glucose, random blood glucose, and oral glucose tolerance tests. An HbA1c level of <5.7% is considered normal, 5.7% to 6.4% indicates prediabetes, and >6.5% indicates diabetes. A fasting blood glucose value of <100 mg/dl is normal, 100 to 125 mg/dl indicates prediabetes, and ≥126 mg/dl in two separate tests indicates diabetes. In a random blood glucose test, the blood glucose value ≥200 mg/dl indicates diabetes. In a less commonly used oral glucose tolerance test, 2-hour plasma glucose ≥200 mg/dl after a 75-gram load of glucose indicates diabetes (1).

In cases where it is ambiguous to determine the type of diabetes, measurement of diabetes-related autoantibodies can help to distinguish between T1DM and T2DM. People above 40 years of age, newly diagnosed with diabetes, and responding well to oral glucose-lowering medicines usually do not require antibody testing. Moreover, HbA1c is not recommended as a diagnostic test in T1DM as it does not capture a recent sudden increase in blood glucose levels (22).

1.6. Management of diabetes mellitus

1.6.1. Type 1 diabetes mellitus

People with T1DM require external insulin to survive and maintain their glucose levels in the desired range. Insulin has four types depending on their rate of action, peak time, and duration of action. These include rapid-acting, regular- or short-acting, intermediate-acting, long-acting, and ultra-long-acting insulin. Rapid-acting insulin reaches the bloodstream within 15 minutes of administration, has a peak time of 1–2 hours, and lasts 2–4 hours. Short-acting insulin reaches the bloodstream within 30 minutes of administration, peaks at 2–3 hours, and lasts 3–6 hours. Intermediate-acting insulin (NPH insulin) reaches the bloodstream within 2–4 hours of administration, peaks at 4–12 hours, and lasts 12–18 hours. Long-acting insulin reaches the bloodstream after several hours of administration and lowers glucose levels for 24

hours. Ultra-long-acting insulin reaches the bloodstream within 6 hours of administration and lasts almost 36 hours (23).

Insulin can be administered either by subcutaneous multiple daily injections (MDI) or by continuous insulin infusion devices, depending on the type of required insulin therapy, patient needs, and reimbursement policies (23).

Regular glucose monitoring is required to administer and adjust the insulin dose accordingly, so people can live healthy lives and prevent acute and chronic complications of T1DM. In addition to insulin, physical activity and a healthy diet play significant roles in managing T1DM. Since T1DM predominately occurs in children and adolescents, it is difficult for them to adhere strictly to the self-management plan (18).

1.6.2. Type 2 diabetes mellitus

The management of T2DM requires a multidisciplinary approach that includes a healthy diet, regular physical activity, managing risk factors, adhering to treatment, and regular follow-ups. Usually, the first line of management involves lifestyle modification for managing blood glucose. If lifestyle modification is unsuccessful, treatment is started with oral glucose-lowering medication, and metformin is usually the first choice. In case a single glucose-lowering medication cannot achieve glycaemic control, various combination therapies of oral glucose-lowering medicines are available. These classes of oral glucose-lowering medicines include sulfonylureas, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase 4 [DPP-4] inhibitors, glucagon-like peptide 1 [GLP-1] agonists, and sodium-glucose co-transporter 2 (SGLT-2) inhibitors. Moreover, Insulin can also be used in combination with oral glucose-lowering drugs if glycaemic control is not achieved with oral medicines alone (18).

In addition to blood glucose management, clinical guidelines recommend screening and managing people with T2DM for known coexisting conditions like hypertension and dyslipidaemia. Also, people should be regularly screened for microvascular and macrovascular complications like chronic kidney disease (CKD), retinopathy, neuropathy, peripheral vascular disease, and foot ulcers to implement timely and effective preventive and treatment strategies (18).

1.7. Complications of diabetes mellitus

1.7.1. Type 1 diabetes mellitus

Acute and potentially life-threatening complications of T1DM include hypoglycaemia and DKA. The occurrence rate of a severe hypoglycaemic event requiring others' assistance is 16–20 per 100 person-years. The rate of a hypoglycaemic event resulting in the loss of consciousness or seizure is 2–8 per 100 person-years (24). Hypoglycaemic events are associated with an increased risk of cognitive dysfunction and 4–10% of T1DM-related deaths (24). The incidence of DKA ranges from 0 to 56 per 1000 person-years, and its prevalence ranges from 0 to 128 per 1000 people (25). The in-hospital treatment rate for DKA in children is reported between 1 to 10 per 100 patient-years, and DKA-associated mortality is reported between 13% to 19% (24).

Chronic complications of T1DM are classified as microvascular and macrovascular complications. Microvascular complications of T1DM include retinopathy, neuropathy, and nephropathy. The estimated prevalence of diabetic retinopathy in T1DM is 80%. In addition, the risk of developing macular oedema, cataracts, and glaucoma is high in T1DM. Diabetic nephropathy is recognized as the foremost cause of CKD. Evidence indicates that controlling HbA1c through appropriate management reduces the incidence and progression of microvascular complications by 37–76% (24,26).

Common macrovascular complications of T1DM include cardiovascular disease (CVD), peripheral vascular disease, lower limb amputations, and cognitive impairment. CVD is a major macrovascular complication of T1DM and has been found to reduce the life expectancy of people with T1DM by 8–13 years compared to healthy counterparts. Evidence suggests that strict blood glucose control can effectively reduce the risk of cardiovascular complications by 40–58% (24,26). People with T1DM have an 85-fold higher risk for lower limb amputations than those without T1DM. T1DM also increases the risk of acute and chronic neurocognitive impairments, which are caused by acute, microvascular, and macrovascular complications (24,26).

1.7.2. Type 2 diabetes mellitus

Like T1DM, complications of T2DM are classified as acute and chronic (macrovascular and microvascular) complications. However, acute complications of hypoglycaemia and DKA occur more frequently in people with T1DM than T2DM.

Microvascular complications that develop primarily due to hyperglycaemia are widespread in T2DM. Diabetic nephropathy and retinopathy are prevalent in 25% of people with T2DM, diabetic neuropathy in 50%, and erectile dysfunction in 35–90% of men with T2DM (27). The risk of microvascular complications is 10–20 times greater in people with T2DM than those without T2DM. In middle- and low-income countries, the median prevalence of retinopathy, nephropathy, and neuropathy were 12%, 15%, and 16%, respectively. In the United States, the incidence rate of peripheral neuropathy is 26.9 per 1000 person-years, CKD 21.2 per 1000 person-years, and CVD 11.9 per 1000 person-years (28).

Common macrovascular complications of T2DM that develop due to atherosclerosis, insulin resistance, and hyperglycaemia include CVD and lower limb amputations. The risk of macrovascular complications is 2–4 times greater in people with T2DM than those without it. People with T2DM develop CVD on average 15 years earlier than people without T2DM. Moreover, the risk of developing CVD and dying from vascular diseases in T2DM is approximately two times higher than in non-T2DM. The incidence of lower limb amputations in T2DM ranges between 78 and 704 per 100,000 person-years, with a relative risk of 7.4 to 41.3 (28,29).

1.8. Influenza

1.8.1. Overview of influenza virus

The influenza virus consists of a single-stranded RNA and belongs to the Orthomyxoviridae family. It is classified into A, B, C, and D types based on its core proteins. It infects both humans and animals and is naturally present in aquatic birds. It is further classified into several strains according to combinations of its hemagglutinin (HA) and neuraminidase (NA) surface proteins. The strains of influenza A currently circulating worldwide are H1N1 and H3N2. Influenza B virus infects only humans. It is divided into Yamagata or Victoria lineages according to differences in the haemagglutinin glycoproteins. Both influenza A and B are common causes of respiratory infections in humans; however, only influenza A viruses have caused major epidemics and pandemics thus far. Influenza C is a less common virus that infects both humans and swine. It usually causes mild respiratory infection and therefore is not considered a public health priority. Influenza D mainly infects pigs and cattle with no evidence of infecting humans (30,31).

The influenza virus core contains the viral genome, which is composed of eight single-stranded RNA segments. The segmented genome encodes ~14 proteins that include surface proteins, matrix (M) proteins, nuclear protein (NP), nuclear export protein (NEP), non-structural proteins (NS), polymerase acid proteins (PA), and polymerase basic proteins (PB). PA, PB, and NP are required for assembling the viral ribonucleoprotein (vRNPs) and synthesizing the viral RNA. The viral genome is encapsulated by a lipid bilayer cell membrane, which is embedded with two glycoproteins HA and NA. The M1 protein supports the viral genome by forming a matrix inside the cell membrane. Also, M1, along with NEP, mediates the nuclear export of the viral genome. The M2 is a transmembrane protein that serves as an ion channel and is dispersed throughout the cell membrane. The NS1 contributes to inhibiting the immune response of the host during infection (30,31).

1.8.2. Pathophysiology of influenza

In humans, the influenza virus causes respiratory infection by directly infecting the respiratory epithelium. The virus can also infect other cell types, such as immune cells. However, the most effective site for cleaving the HA protein of the virus in humans is the respiratory epithelium. The virus attaches its HA protein to the sialic receptors on the cell surface and enters the cell through the process of receptor-mediated endocytosis. The low pH (5–6) in the endosome mediates the release of the viral genetic material into the cytoplasm of the host cell by uncoating the viral membrane. Afterward, the viral NP recognizes and binds the viral genome to the host nuclear import machinery for translocating it to the nucleus. In the nucleus, the PB protein facilitates the transcription and assembly of new vRNPs. The viral M1 and NEP, with the assistance of PA and PB proteins, transport these transcripts to the cell cytoplasm for protein synthesis. The viral segments assemble at the host cell membrane, and a newly generated virus exits the cell to infect adjacent cells. Meanwhile, the immune system responds to the infection by inflaming cells to manage the spreading virus. This inflammation can spread into the lungs and systemic circulation and cause multiorgan failure and death (31,32).

Influenza eludes the immune system by altering its antigenic characteristics via two types of mutations – antigenic drift or antigenic shift. In antigenic drift, small mutations occur in the virus's genes during its replication. These changes are usually too small to alter the antigenic properties of the virus. However, these mutations accumulate over time, and the virus evolves by producing different HA and NA surface proteins. As surface proteins of the virus serve as antigens for the immune system to trigger an immune response, new surface proteins are not recognized by the host immune system, resulting in infection. Antigenic shift

is a major and abrupt mutation in the influenza virus. Research suggests that the segmented genome of the virus allows easy antigenic shift. Antigenic shift occurs when an influenza virus is transmitted from animals to humans and causes infection. Mixing of these animal and human influenza viruses causes genetic reassortment, resulting in the emergence of novel variants of influenza viruses. These variants produce surface proteins that are not recognized by the host immune system. Hence, humans possess little or no immunity against these variants (31,32).

Influenza viruses evolve continuously due to antigenic drifts, whereas antigenic shift occurs rarely. Influenza A viruses mutate by both antigenic drift and antigenic shift, while influenza B viruses only mutate by antigenic drift. Moreover, antigenic drift occurs more rapidly in Type A than in Type B viruses. The antigenic shift of influenza A causes epidemics and pandemics, while influenza variants produced by antigenic drift cause seasonal epidemics annually (31,32).

1.8.3. Transmission, clinical presentation, and complications of influenza

The influenza virus mainly spreads via respiratory droplets. It is transmitted by coughing, sneezing, even normal breathing, or direct contact with infected people or surfaces. The usual incubation period is 18 to 72 hours, with some variability. Most influenza infections are asymptomatic. Common clinical manifestations of symptomatic influenza include the sudden onset of fever, rhinitis, headache, cough, sore throat, fatigue, and muscle aches. In adults, fever usually lasts 3 days, while other symptoms can last 7 to 10 days. Some patients may also develop ocular symptoms such as photophobia, watery eyes, and pain and redness in the eyes. Pneumonia is the most frequent complication of severe influenza infection, which develops due to the influenza virus or secondary bacterial infection. In rare cases, influenza infection can lead to serious complications such as lung injury, acute respiratory distress syndrome (ARDS), cardiac complications, neurological infections, and even death (33).

1.8.4. Epidemiology of influenza

Influenza is a widespread infection that contributes to significant morbidity and mortality worldwide – resulting in significant healthcare and economic burden and loss of functionality and productivity in life. Every year, different strains of influenza are estimated to infect up to 10–15% of adults and 20–30% of children, accounting for 1 billion cases worldwide (34,35). According to the global influenza report, the attack rate of influenza was 19% between November 2019 and December 2020. Of these positive influenza cases, 63% were influenza A and 37% were influenza B. Within influenza A, 23% were H1N1, 17% were H3N2, and 61%

were not of subtype A. Within influenza B, 16.2% had Victoria lineage, 0.3% Yamagata lineage, and 83.5% were not subtyped (36). Annually, influenza is associated with approximately 3–5 million cases of severe illness and 290,000–650,000 deaths worldwide. Of these deaths, 72,000 occur in Europe (35). In the United States of America, 35,000,000 cases of illness, 380,000 hospitalizations, and 20,000 deaths associated with influenza were reported in 2019–2020 (34).

Influenza circulates in the population as seasonal flu throughout the year and peaks during different seasons depending upon the geographic region. In temperate regions, seasonal epidemics of Influenza mostly occur during months of winter, while it occurs sporadically throughout the year with irregular outbreaks in tropical and subtropical regions. In the Northern hemisphere, influenza season begins in October and peaks in January or February. In the Southern hemisphere, influenza season begins in May and peaks between June and August in different countries (37).

Various strains of influenza A have caused several pandemics in previous and current centuries. In 1918, the Spanish flu pandemic, originated in Spain and caused by H1N1, killed around 50 million people worldwide. In 1957, the Asian flu pandemic, originated in China and caused by H2N2, killed around 4 million people worldwide. In 1968, the Hong Kong flu, caused by H3N2, killed nearly 1 million people worldwide. The bird flu was caused by H5N1 in 2005 and Swine flu by H1N1 in 2009 (38).

Influenza causes mild illness in most instances; however, it can cause severe illnesses such as pneumonia and ARDS in high-risk groups, thereby leading to hospitalization and even death. These high-risk groups include children under 59 months, pregnant women, the elderly, healthcare workers, and people with underlying serious chronic and immune-compromised conditions such as CVD, chronic respiratory diseases, asthma, and diabetes (33,39). As per the global disease burden study, geographic regions, children under 5 years, and adults older than 75 years are significant risk factors for influenza-associated respiratory death annually (39,40).

1.9. COVID-19

1.9.1. Overview of COVID-19

Coronaviruses are members of the Coronaviridae family and infect both animals and humans. In humans, four coronaviruses are known to cause infections that range from

common cold to severe respiratory syndrome. These viruses are HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1. Two coronaviruses of zoonotic origin named as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have caused major outbreaks in the past (41).

The SARS-CoV-2 is a novel coronavirus, which was discovered in 2019, and causes coronavirus disease (COVID-19). The core of coronavirus consists of tightly packed single-stranded RNA polymers, which are surrounded by a protective shell of capsid proteins also called nucleocapsid (N). The central core is further encapsulated by an outer lipid bilayer membrane, which consists of lipids, structural proteins such as spike (S), membrane (M), and envelope (E) proteins that are embedded into the outer membrane, and many non-structural proteins (nsp). The N-protein participates in the replication and transcription of the virus. The M-protein is the most abundant protein on the viral membrane and is responsible for assembling the virus. The S-protein protrudes from the outer membrane to give it a crown-like shape and attaches the virus to the surface receptors of host cells for entry. The S-protein has two functional subunits referred to as S1 and S2. The S1 is responsible for binding the virus to the angiotensin-converting enzyme 2 (ACE2) receptor of the host cells via a fragment referred to as the receptor-binding domain (RBD). The S2 facilitates the merging of viral and host cell membranes. The E-protein is a small protein that facilitates assembly and membrane permeability of the virus and virus-host cell interaction (42,43). Since the discovery of the SARS-CoV-2 virus, it has undergone several mutations in its S-protein to produce new variants. Common variants of the SARS-CoV-2 virus include Alpha, Beta, Gamma, Delta, and Omicron (44).

1.9.2. Pathophysiology of COVID-19

COVID-19 is primarily an infection of the upper respiratory tract. At first, the virus enters the upper respiratory system and attaches itself to ACE2 receptors of the host cell via the S1 subunit of the S-protein. These ACE2 receptors are located on the surfaces of many cells and are overexpressed in respiratory and pulmonary epithelial cells. The host proteases such as trypsin and furin cleave the S-protein. The cleavage process stabilizes the S-protein, and the virus enters the host cell by endocytosis or membrane fusion, which is facilitated by the S2 subunit of S-protein. After entering the alveolar epithelial cell, the virus releases its RNA into the cytoplasm. Inside the infected host cell, the transcription and replication of viral RNA occurs, which is facilitated by the nsp. Afterward, the new viral RNA synthesizes structural proteins, the N protein binds the new RNA, while the M protein facilitates its integration to the cellular ER. Next, the newly generated nucleocapsids are exported to the cell membrane via

Golgi vesicles and exit the cell via exocytosis. Thereon, the new viral components can infect neighboring epithelial cells as well as transmit to other people via respiratory droplets. At the same time, the ER stress induced by viral replication leads to cellular apoptosis (42,43,45).

1.9.3. Stages of COVID-19 infection

COVID-19 infection has three stages. The first stage is an asymptomatic phase. In this phase, the virus replicates and spreads locally in nasal epithelial cells and conducting pathways. This phase usually lasts two days and the host immune response is minimal. However, people are infectious, and the virus can be detected via a nasal swab. In the second phase, the virus migrates from the nasal epithelium to the upper respiratory tract. During this stage, symptoms of fever, dry cough, and malaise develop. The immune response also increases during this phase and C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFN-β and IFN-λ) are released from the infected cells. Most patients (80%) do not get past this phase as the increased immune response can contain the infection. Moreover, this stage lasts for 10–14 days. In the third stage, the infection progresses to the lower respiratory tract and may cause ARDS. Approximately 20% of infected people develop this stage and manifest severe symptoms. Specifically, the virus enters epithelial cells of alveoli and undergoes the process of transcription and replication to generate more nucleocapsids. In response, the host immune system is hyperactivated and infected alveolar cells release various cytokines and interleukins (IL-1, IL-6, IL-8, IL-120, and IL-12), TNF-α, IFN-λ, IFN-β, CXCL-10, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α), referred to as a cytokine storm. The cytokine storm attracts neutrophils and CD4+ and CD8+ T cells in the lungs. These cells fight against the virus; however, in doing so, they also inflame and injure the lungs. As a result of viral replication, the virus continues to infect neighboring alveolar epithelial cells. The viral replication and inflammatory cells damage alveolar epithelial cells, eventually leading to ARDS (42,43,45).

1.9.4. Transmission, clinical presentation, and complications of COVID-19

The primary transmission mode for the SARS-CoV-2 virus is respiratory droplets. The virus transmits by coughing, sneezing, or touching infected surfaces. Airborne transmission has not been documented yet except during the procedures that generate aerosols. Its usual incubation period is 5–6 days but can be up to 14 days. Common clinical manifestations of COVID-19 are fever, headache, body aches, breathlessness, malaise, dry cough, and loss of smell and taste. In some cases, people develop gastrointestinal symptoms such as abdominal pain, vomiting, and diarrhea (43). COVID-19 affects multiple body systems and leads to

various debilitating pulmonary and extrapulmonary complications. Its most common pulmonary complications are pneumonia, lower respiratory tract infections, ARDS, respiratory failure, and pneumothorax. Common extrapulmonary complications include arrhythmias, acute cardiac injury, heart failure, acute kidney injury (AKI), coagulopathy, acute liver injury, multi-organ failure, and death (46).

1.9.5. Epidemiology of COVID-19

COVID-19 emerged in Wuhan, China, in December 2019. Since its emergence, it has spread rapidly worldwide and was declared a pandemic by the world health organization (WHO) (47). As of July 2022, approximately 566,977,818 confirmed cases of COVID-19 and nearly 6,376,503 deaths related to this pandemic have been reported worldwide (47). However, these numbers are underestimated because of a high proportion of people with asymptomatic infection and issues in reporting cases, particularly in low-income countries. A high proportion of COVID-19 cases have been shown to develop severe disease and various complications and experience mortality. A global meta-analysis comprising 6007 studies estimated that nearly one-fourth (23%) of people developed severe COVID-19 infection, and the overall fatality rate following COVID-19 was 5.6%. A systematic analysis in 2020 and 2021 estimated that the global excess mortality rate attributed to COVID-19 was 120.3 per 100,000 population (48). Due to the high morbidity, severity, and mortality rate of COVID-19, the WHO has declared it a public health emergency (49).

The disease burden of COVID-19 differs significantly among geographic regions and countries. With respect to geographic regions, 238,767,709 cases of COVID-19 and 2,043,132 deaths have been reported in Europe, 168,183,683 cases and 2,779,572 deaths in Americas, 69,359,556 cases and 243,503 deaths in Western Pacific, 59,187,567 cases and 791,834 deaths in South-East Asia, 22,490,905 cases and 344,525 deaths in Eastern Mediterranean, and 9,187,634 cases and 173,924 deaths in Africa (47). Consistent with these crude numbers, the mortality rates following COVID-19 differ considerably among regions. The excess death rate was the highest in South Asia, followed by North Africa, the Middle East, and Eastern Europe. A comparison among countries showed that the number of cumulative excess deaths due to COVID-19 was the highest in India (4.07 million), followed by the United States (1.13 million), Russia (1.07 million), Mexico (798,000), Brazil (792,000), Indonesia (736,000), and Pakistan (664,000). The pooled mortality rate was 14.3% in Italy, 5.3% in China, 4.4% in the United States, and 0.9% in South Korea (49).

Many factors have been identified to increase the risk of COVID-19 morbidity, severity, and mortality. Older adults, males, obesity, people with underlying chronic conditions such as compromised immune system, diabetes, cancer, CVD, chronic respiratory diseases, chronic liver disease, chronic kidney disease, and certain ethnicities are considered more vulnerable to COVID-19 and its related mortality (49,50). While older adults and those with underlying chronic conditions such as immunocompromised diseases, diabetes, chronic respiratory diseases, CVD, and cancer are more likely to experience a severe course of COVID-19 infection. Also, people experiencing gastrointestinal symptoms (nausea, vomiting, and abdominal pain) and respiratory symptoms (chest pain, shortness of breath) are more likely to develop a severe infection, whereas people with pneumonia and end-organ failure have an increased risk of COVID-19-related mortality (49).

The emergence of various SARS-CoV-2 variants has significantly influenced its transmission, disease outcomes, and re-infection rate. The Alpha variant (identified in the United Kingdom) spread rapidly worldwide, exhibited a 50–75% faster rate of transmission than previous strains, and was associated with higher disease severity. Although less prominent, the Beta variant (identified in South Africa) possessed the properties of evading the host immune system. The Gamma variant (identified in Japan), also not a prominent variant, had a rapid transmission rate and was virulent. The Delta variant (identified in India) was the most common variant globally and demonstrated a higher transmission, disease severity, and hospitalization rate than the Alpha variant. The latest variant is Omicron and its sublineages (identified in Botswana and South Africa). This variant increased the SARS-CoV-2 infection and re-infection rates and rapidly spread in other countries (44).

1.9.6. COVID-19 biomarkers

COVID-19 was initially considered only a respiratory infection with pulmonary complications like pneumonia and ARDS in severe cases. In light of evolving evidence, it is now recognized as a systemic disorder in which multiple body systems are affected by a diffused systemic pathophysiological process involving immunological, inflammatory, and coagulation cascades interacting in a complex manner (51). These pathological processes may disrupt serum concentrations of various biomarkers of involved systems depending upon the phase and severity of SARS-CoV-2 infection. Hence, laboratory measurements of such biomarkers may offer many benefits for people suffering from COVID-19: 1) detection of vulnerable patients; 2) stratification of disease severity; (3) triaging patients for intensive care; (4) optimization of the treatment plan; 5) evaluation of prognosis; 6) and monitoring and evaluation of patients' response to therapeutic interventions (51). In this perspective, various

system-specific prognostic biomarkers have been investigated and identified for COVID-19 that are discussed below:

Inflammatory biomarkers

The SARS-CoV-2 attacks alveolar epithelial cells, activating both innate and adaptive immune systems. The hyperactivation and dysregulation of proinflammatory and immunological responses lead to the development of cytokine storm. Consequently, various proinflammatory cytokines such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-10, IL-17, TNF-α, and interferons are released. Excessive release of cytokines mediates the recruitment of macrophages, T cells, and neutrophils to the infected site. These dysregulated immune responses destabilize endothelial cell-to-cell interactions, damage vascular barriers and tissues, and ultimately lead to multiple organ failure. Research has implicated IL-6 as a pivotal proinflammatory biomarker of prognosis, severity, and treatment response to COVID-19. Other proinflammatory cytokines such as IL-1β, IL-2, IL-8, IL-17, G-CSF, GMCSF, inducible protein-10, and monocyte chemoattractant protein-1 have also been found elevated in seriously ill COVID-19 patients (51–54).

Production of serum cytokines and TNF-α trigger several immunological pathways, which in turn promotes the synthesis of C-reactive protein (CRP). CRP, synthesized by the liver, is a well-established sensitive albeit non-specific and acute biomarker of inflammation, infection, and tissue damage. Studies suggest that elevated levels of serum CRP in COVID-19 correlate well with severe disease, adverse events, and in-hospital mortality (51–54).

Procalcitonin (PCT), a precursor of calcitonin, is used as a reliable biomarker for detecting bacterial infections and guiding antibiotic therapy. However, research suggests that during SARS-CoV-2 infection, serum cytokines and TNF-α mediated immunological pathways promote the synthesis of PCT. Moreover, increased concentrations of PCT have been strongly correlated with COVID-19 severity, suggesting its potential role as a biomarker in this viral infection as well as an indicator of coexisting bacterial infection (51–54).

Ferritin is a cellular protein that regulates iron metabolism. It is also an inflammatory protein of an acute phase that increases during inflammatory conditions, coronary artery disease, and cancer. In addition, ferritin has already been identified as a significant prognostic factor for ARDS. Similarly, some studies have also claimed that elevated levels of ferritin indicate COVID-19 severity (51–54).

Given the incriminating translational and clinical evidence, elevated serum levels of cytokines, CRP, PCT, ferritin, and LDH can be used as reliable prognostic biomarkers for COVID-19.

Coagulation biomarkers

Hypercoagulation has been identified as a unique manifestation of severe COVID-19, which could lead to thromboembolic events such as deep vein thrombosis, pulmonary embolism, and microvascular thrombosis. Endothelial dysfunction and damage can occur by viral invasion or dysregulated adaptive immune response. The immune-triggered thrombosis causes vascular damage, systemic microangiopathy, and thromboembolism, which may lead to multi-organ failure. In addition, disseminated intravascular coagulopathy induces massive microvascular thrombosis. COVID-19-induced coagulopathy has significantly altered various coagulation biomarkers, which have been identified as significant biomarkers of COVID-19 severity and prognosis (51,52,54).

D-dimer is a degradation product of soluble fibrin that has long been used as a biomarker for diagnosing thromboembolic events such as pulmonary embolism. Increased serum concentrations of D-dimer have been shown to increase the likelihood and risk of severe COVID-19 and related in-hospital mortality. Therefore, it may serve as a reliable prognostic marker for COVID-19 (51,52,54). Also, prothrombin time (PT), D-dimer, and fibrin/fibrinogen have been significantly correlated with COVID-19 severity, ARDS development, and in-hospital mortality. Given its clinical usefulness, it has been recommended to triage patients into critical care. The platelet count is also considered a reliable biomarker for disease severity and is significantly decreased in patients with severe COVID-19 compared with those with mild COVID-19 (51,52,54).

Haematological biomarkers

COVID-19 disrupts the hematopoietic system and haemostasis, which alters concentrations of several haematological cells. White blood cells are considered valuable indicators of inflammation, severity, and prognosis of infections. Lymphocytopenia is a hallmark of COVID-19 that develops due to apoptosis of lymphocytes mediated by the SARS-CoV-2 virus and its antibodies, cytokine storm, and coexisting lactic acidosis. Therefore, it has been suggested as a reliable biomarker for evaluating COVID-19 severity and prognosis (51,52,54). Apart from lymphocytopenia, monocytes, eosinophils, and basophils counts have been found significantly decreased in non-survivors compared to survivors of COVID-19.

Conversely, studies have shown that, like in many infections, the neutrophil count increases substantially during COVID-19. During an infection, neutrophils migrate from the blood into highly vascular tissues such as lungs and kidneys. This influx can potentially damage adjacent blood vessels and parenchyma. Neutrophils also release neutrophil extracellular traps (NETs) upon facing threat signals, which may cause vascular injury and autoimmune vasculitis. Therefore, the neutrophil count can serve as a valuable biomarker for COVID-19 severity (51–54). However, compared to individual haematological biomarkers, accumulating evidence suggests that the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) offer better performance for determining the severity and prognosis of COVID-19 (51–54).

Hepatic biomarkers

COVID-19 has been reported to cause liver dysfunction and acute liver injury by directly damaging biliary and hepatic endothelial cells, immune-mediated damage of hepatic cells, or sustained hypoxia of hepatic cells. The non-COVID-19 causes of liver injury include hepatotoxic antiviral and antibiotic medicines used for treating COVID-19. The extent of liver dysfunction and damage is reflected by increased levels of enzymes like alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin levels in the bloodstream. Therefore, measurement of liver enzymes can provide information about the severity and prognosis of COVID-19 (52,55).

Hypoalbuminemia also occurs frequently during COVID-19, which could result from liver dysfunction caused by direct viral damage or cytokine storm. Clinical and epidemiological studies have also reported that hypoalbuminemia occurred most frequently in COVID-19 patients who died (82%), followed by those who were seriously ill (71%), while it was the lowest in those with mild disease (38%). In addition, hypoalbuminemia was associated with poor prognosis measured as the duration of hospital stay and mortality (56,57).

Cardiac biomarkers

Cardiovascular dysfunction also referred to as a cardiovascular syndrome, is the most frequent and serious extrapulmonary complication of COVID-19. Cardiovascular syndrome comprises acute coronary syndrome, myocardial injury, decompensated heart failure, stress-induced cardiomyopathy, pericardial effusion, viral myocarditis, or arrhythmia. This syndrome develops because of various complex pathophysiological mechanisms. Myocardial injury may result from direct viral invasion into myocardial cells via ACE2 receptors, dysregulated immune

response, cytokine storm, coagulopathy, or accompanying haemodynamic derangements (58). In addition, these systemic derangements along with pulmonary complications exert stress on the myocardium to maintain cardiac function, which inflicts further ischemic injury to the myocardium and results in the elevation of cardiac biomarkers such as troponins, creatine kinases, and natriuretic peptides. Moreover, myocardial injury further activates immune and inflammatory responses, which may elevate disease severity and mortality. Clinical and epidemiological data strongly support the development of myocardial ischemia, injury, and other related complications following SARS-CoV-2 infection. In addition, COVID-19 seriously compromises the cardiovascular system of people with existing CVD and aggravates their prognosis (51,52,54).

Cardiac troponins such as troponin T and troponin I and creatine kinase-myocardial band (CK-MB) are well-established sensitive and specific biomarkers for myocardial injury. Troponin I has also been identified as a valuable predictor of mortality in various respiratory diseases and sepsis. As cardiac injury is a frequent complication of COVID-19, cardiac troponins and CK-MB have been identified as valuable biomarkers for determining disease severity and mortality in COVID-19 patients (59,60). Also, patients with high troponin I levels at presentation required assisted ventilation and developed ARDS more frequently than those with its normal levels (51,52,54).

Plasma natriuretic peptides (NPs) such as brain type NPs (BNP) and N-terminal pro-BNP (NT-proBNP) are established biomarkers for myocardial stress and therefore are used for evaluating the prognosis of heart failure. Evidence suggests that people with existing heart failure are more prone to experience severe COVID-19 and its adverse outcomes. On the other hand, COVID-19 has been proposed to induce heart failure secondary to ARDS, myocarditis, and ischemia. Therefore, increased levels of BNP and NT-proBNP have been detected in COVID-19 patients with and without existing heart failure. In addition, elevated NT-proBNP levels and cardiac troponins have been found associated with higher in-hospital mortality rates with excellent predictive performance in COVID-19. While higher levels of BNP have been correlated with cardiogenic pulmonary oedema in COVID-19 patients with ARDS (51,52,54).

Renal biomarkers

AKI is another common complication of COVID-19. Studies show that approximately 30–50% of hospitalized COVID-19 patients develop AKI. In addition, nearly 50% of patients with AKI in intensive care units require renal replacement therapy and in-hospital mortality is higher in AKI patients. Furthermore, patients with existing chronic kidney disease are also

more susceptible to severe COVID-19 and mortality. Underlying mechanisms that are proposed to cause AKI include local or systemic inflammatory responses, direct or indirect endothelial injury to renal cells, abnormal coagulation processes, and renin-angiotensin system activation. The AKI is measured by routinely measured estimated glomerular filtration rate (eGFR) and serum creatinine, and therefore can be used as feasible biomarkers for COVID-19 (52,54).

Other biomarkers

Other biomarkers that have been found significantly associated with COVID-19 severity and prognosis include Lactate dehydrogenase (LDH) and Serum amyloid A (SAA). LDH is an enzyme present in all human cells and regulates the production and secretion of lactate. Lactate regulates various biological processes such as inflammatory response modulation, innate immunity suppression, and T helper cell differentiation. In the SARS-CoV-2 infection, systemic inflammatory response and coagulopathy promote vascular damage and cause persistent hypoxia, which alters glucose and DNA metabolism. Clinical and epidemiological evidence also shows that COVID-19 patients admitted to ICU have significantly higher LDH levels than non-ICU patients and LDH levels are positively correlated with tissue damage. Furthermore, LDH levels have been found significantly elevated in patients requiring mechanical ventilation and concomitant corticosteroid and antiviral therapy. Therefore, LDH is a valid biomarker for evaluating COVID-19 disease severity and mortality (51,52,54).

Serum amyloid A (SAA) belongs to a family of apolipoproteins, which is activated in response to acute inflammation, infection, and changes in hormone metabolism. During the acute phase, TNF, interleukins, and interferons trigger the synthesis of SAA in the liver, and its levels can increase up to a thousand times within first 24 to 48 hours. This rise in SAA levels can further increase the synthesis of proinflammatory markers, which contribute to the development of cytokine storm in later phases of the SARS-CoV-2 infection. It is therefore plausible that elevation in SAA levels not only reflects the acute phase of SARS-CoV-2 infection but also predicts disease progression and subsequent adverse outcomes. Clinical evidence also supports that SAA has a reasonable ability to distinguish between mild and severe cases of COVID-19. A meta-analysis has also reported higher concentrations of SAA in severe COVID-19 cases and non-survivors compared to mild cases and those who survived (52,61).

1.9.7. Prognostication of COVID-19

COVID-19 is a debilitating disease with high severity, complication, and mortality rates (47). An estimated one-fifth of people with COVID-19 develop a serious illness and complications that often require hospitalization for appropriate management (48). Among those hospitalized, approximately one-fourth need intensive care and assisted ventilation (62). The high burden of severe COVID-19 has put an enormous strain on resources and service delivery of health systems to effectively manage hospitalized and seriously ill patients with COVID-19 (63). In this health crisis, illness severity prognostication scores can provide a cost-effective, feasible, and practical strategy for stratifying high-risk patients to appropriately allocate healthcare resources, guide treatment plan, monitor disease progress, and evaluate the effectiveness of treatment (64).

Various prognostication scores have been developed worldwide to monitor the quality of care, stratify high-risk patients, and evaluate their prognosis in critical care settings (64). The Confusion, Urea, Respiratory rate, Blood pressure, and age above or below 65 years (CURB-65) is an internationally validated score for predicting all-cause mortality within 30 days among patients diagnosed with community-acquired pneumonia (65). The National Early Warning Score 2 (NEWS2) is an early warning tool to detect acute deterioration in patients' health (66). The Quick Sequential Organ Failure Assessment (qSOFA) score predicts mortality and ICU admission among patients with suspected infection prior to ICU admission (67). The Acute Physiology and Chronic Health Evaluation II (APACHE-II) score estimates the mortality among patients admitted to ICU (68). The Mortality Probability Model 0–III (MPM0-III) evaluates the mortality among ICU patients (69). However, most of these prognostication scores are not disease-specific and consist of parameters reflecting only the severity of a clinical condition for predicting adverse outcomes. In addition, these scores require frequent validation and recalibration to adjust for advancements in diagnostic and therapeutic procedures and evolution in the ICU practice and disease trends (64).

The simplified acute physiology score version 3 (SAPS 3) is a widely used prognostication score for critically ill patients admitted to the ICU with any underlying disease. It was developed from a multicenter study conducted in 35 countries. It comprises parameters ranging from patient characteristics, physiological and laboratory measurements, and ICU-related factors that are accumulated to evaluate disease severity, monitor the quality of care, and predict in-hospital mortality. It has been extensively validated in various regions, healthcare settings, and patients with different diseases and has exhibited satisfactory

performance in most instances. In addition, the SAPS 3 has both global and region-specific equations to yield optimal predictive performance for these regions (70).

The SAPS 3 is being utilized in many countries, including Austria, as a part of routine ICU care to monitor and evaluate the disease severity and prognosis of COVID-19 patients. Although SAPS 3 has demonstrated satisfactory predictive performance in various disease cohorts, it is strongly recommended to validate and recalibrate this tool before utilizing it in a desired patient population (64). Some studies have evaluated the performance of SAPS 3 in COVID-19 patients. In Brazilian COVID-19 patients, the SAPS 3 demonstrated satisfactory discrimination with an area under the curve (AUC) of 84%; however, its calibration was inadequate as the mortality was underestimated in lower to intermediate risk groups. Moreover, even recalibration of the score could not address this issue (71). Likewise, a previous study conducted during the first wave of COVID-19 in Austria reported that the SAPS 3 had good discrimination. However, consistent with a Brazilian study, in-hospital mortality was underestimated by this tool, particularly in lower-risk strata. Therefore, the SAPS 3 equation was customized to yield better calibration (72). Moreover, another study among Brazilian COVID-19 patients found that the SAPS 3 performed well in COVID-19 patients in both first (AUC = 90%) and second (AUC = 81%) waves of the pandemic. However, the calibration of SAPS 3 was not examined in this study (73). In addition, a recent study has evaluated the performance of CURB-65, NEWS2, and qSOFA in COVID-19 patients and reported that all these scores underpredicted the mortality in this patient population, which is consistent with the performance of SAPS 3 (74). Two studies have also compared the crude SAPS 3 score with mortality status in COVID-19 patients and reported a higher SAPS 3 score in those who died versus those who survived. However, prediction estimates were not derived in these studies, thus limiting their clinical implications (75,76). The scarcity of evidence and contradictory findings regarding the performance of SAPS 3 in COVID-19 patients suggest that more validation studies are required in COVID-19 patients to establish its clinical utility in this patient population.

1.9.8. Impact of COVID-19 on influenza

The emergence of COVID-19 has significantly impacted the epidemiology of influenza globally. Data show that the incidence of influenza declined drastically by ~99% in 2020 compared to previous years. Only small outbreaks were documented in some tropical countries. In the Northern hemisphere, influenza peaked in late January to early February and persisted till April. In the remaining months, influenza cases occurred sporadically (77). This

reduction is attributed to various factors. As such, the COVID-19 pandemic has drastically changed the personal hygiene practices and health-seeking behaviors of people. Furthermore, quarantine, social distancing, infection control strategies, and travel restrictions on the country and global scale imposed during the COVID-19 pandemic may have contributed to reducing the transmission of influenza. Another possible reason could be the interaction between COVID-19 and influenza viruses, referred to as viral interferences (78). Besides reducing infection rates, the genetic diversity of influenza viruses has also shrunk considerably. For instance, fewer new strains have been detected for H3N2 and H1N1 subtypes and B/Victoria lineage during the COVID-19 pandemic compared to previous years. Likewise, only a few cases of B/Yamagata lineage have been reported globally in 2021 (79).

1.10. Infections and diabetes mellitus

Both T1DM and T2DM are associated with an increased risk of community-acquired and in-hospital respiratory, skin, soft tissue, gastrointestinal, and genitourinary infections. Epidemiological data suggest that leading infections reported at hospitalization in people with diabetes are respiratory, diabetic foot, skin, soft tissue, and urinary infections. Moreover, these infections are more likely to be severe and challenging to treat in the presence of diabetes (80). According to a recent large cohort study, people with diabetes are more susceptible to fungal, viral, and bacterial infections and more likely to have a severe infection and a worse prognosis than those without diabetes (80). In Canada, people with diabetes had a 21% higher risk of outpatient infections compared to those without it. Also, an analysis of one million people in Canada reported that nearly 50% of individuals with diabetes filed an insurance claim for an infectious disease compared to 38% of those without this disease in a cohort year. Concerning in-hospital infections, diabetes contributes to 6–12% of infection-related hospitalizations and 12% of deaths. Diabetes has also been reported to exacerbate the risk of infection-related hospitalization and subsequent death by 2-fold each. Hence, diabetes not only increases the susceptibility to various infections but also contributes to higher hospitalization, ICU admission, and death rates (81).

Both T1DM and T2DM substantially elevate the risk of infections, severe course of the disease, and associated complications by compromising innate and adaptive immune systems at multiple levels. Hyperglycaemia plays a central role in compromising the immune system in diabetes. It impairs the phagocytic function of neutrophils, macrophages, and monocytes by reducing the complement binding to immunoglobulins and impairing the attachment ability of

the complement system to pathogens (80,82). Also, diabetes-induced dysfunction in the complement system impairs polymorphonuclear function and reduces cytokine response. In addition to hyperglycaemia, diabetes induces intrinsic defects in mononuclear cells and monocytes, which decreases the production of interleukins such as IL-1 and IL-6 (82). Moreover, increased glycation in diabetes can reduce the expression of major histocompatibility complex and impair the production of IL-10 by myeloid cells and IFN-γ and TNF-α by T cells, thus impairing cell immunity. Immunoglobulins also get glycated in diabetes, which may impair the biological function of the antibodies. Hyperglycaemia decreases polymorphonuclear leukocyte mobilization, chemotaxis, and phagocytic activity. It also impairs the antimicrobial function by inhibiting glucose-6-phosphate dehydrogenase, increasing the apoptosis of polymorphonuclear leukocytes and reducing their transmigration through the endothelium (82). Moreover, chronic hyperglycaemia in diabetes impairs gastrointestinal and urinary motility, thereby promoting the growth of pathogens. Also, hyperglycaemia significantly elevates the risk of diabetes-related hospitalizations and the duration of hospital stay. Diabetes-induced complications such as neuropathy and poor vascular flow also contribute to excess risk and severity of various infections (83).

1.11. Influenza and diabetes mellitus

Both diabetes and influenza are major causes of illness globally. Data suggest that influenza is the second most common respiratory infection in people with diabetes (82). However, evidence suggests that the relationship between influenza and diabetes is bidirectional in nature. On the one hand, influenza infection has recently been suggested to exacerbate the risk of T1DM. On the other hand, incriminating evidence suggests that people with existing T1DM and T2DM are more susceptible to developing severe influenza infection and its complications than those without these conditions (4).

The association of influenza with the onset of T1DM has been supported by epidemiological, clinical, and animal studies. A study conducted in 1970 reported an increase in the incidence of T1DM and DKA following the influenza epidemic (84). Likewise, another study claimed that the H1N1 pandemic contributed significantly to the onset of T1DM, as indicated by an increase in the number of new cases of T1DM after the pandemic compared to previous years (85). Meanwhile, a study from Norway reported a 20% higher risk of developing T1DM in people with influenza infections. Another analysis from Norway mentioned that the long-term risk of T1DM was 20% higher among people under 30 years who were

diagnosed with influenza during the H1N1 pandemic (86). Likewise, according to a retrospective cohort study in Japan, the risk of developing T1DM was 30% higher 180 days after contracting influenza (87). Consistent with epidemiological studies, a case study from China reported that a woman developed DKA following the H1N1 infection (88). Furthermore, acute pancreatitis was reported by a man after experiencing a severe H1N1 infection (89). Influenza primarily replicates in the respiratory tract; however, invitro, animal, and avian studies have shown that this virus can also replicate in internal organs, including pancreatic islets, which can damage β cells and induce insulitis and autoimmune response, which ultimately leads to the development of T1DM (4).

Diabetes has long been recognized as a major risk factor for severe influenza; however, the majority of evidence concerning this association emerged around the H1N1 pandemic in 2009. A systematic analysis of 93,000 people reported that 14.6% of people with diabetes suffered from severe H1N1 (90). Moreover, prospective studies from various countries have shown that people with diabetes are 2–6 times more likely to be hospitalized, 4 times more likely to be admitted to ICU, and 2 times more likely to die following the H1NI infection (91–94). These findings are further confirmed by a meta-analysis of 234 studies, which estimated that diabetes increased the odds of hospitalization by 4.26 times, severe illness by 1.60 times, and mortality by 2.21 times (39). Due to this convincing evidence, diabetes is regarded as a high-risk condition for viral infections, including influenza.

Several factors contribute to severe influenza infection and its associated complications in diabetes. Recent evidence suggests that persistent hyperglycaemia and acute glycaemic variability, irrespective of HbA1c, may increase the severity of influenza in diabetes (95). While diabetes-induced hyperglycaemia can compromise the immune response by impairing neutrophil degranulation, complement activation, phagocytosis, chemotaxis, and bactericidal activity. Additionally, hyperglycaemia in pulmonary epithelial cells can compromise the structural and functional capacity of pulmonary cells, increase their susceptibility to influenza infection, and enhance the viral replication process (95,96). Regarding acute glycaemic variability, people with diabetes experience more diverse and frequent fluctuations in their glucose levels compared to people without diabetes. Such glycaemic fluctuations can contribute to endothelial dysfunction and apoptosis, overexpression of adhesion molecules, high-mobility group box 1, IL-8, nuclear factor B, and E-selectin. These fluctuation-induced changes in endothelial function also increase oxidative stress, which together with endothelial apoptosis and cytokine production are associated with increased severity of influenza. In addition, oxidative stress can also cause lung lesions and impair antiviral CD8+ T cell responses (95,96). It is important to note that acute glycaemic changes can also be triggered

by influenza infection through dysregulated immune response and induction of cytokine storm. The influenza-induced dysglycaemia has also been suggested to aggravate existing microvascular and macrovascular complications in people with diabetes (96).

Despite diabetes being a high-risk condition for severe influenza and its complications, existing evidence reveals that this relationship differs considerably across studies due to the diabetes population under study, among other factors. The primary reason for such a difference is that diabetes is a complex disease characterized by various demographic, genetic, clinical, and lifestyle-related risk factors, metabolic abnormalities, and multiple concurrent chronic diseases (39). The distribution and prevalence of these characteristics, risk factors, and comorbidities may vary significantly in people with diabetes, making it a clinically diverse population in terms of susceptibility to influenza severity and complications and its prognosis (95,97). For instance, according to a recent large cohort study, men develop diabetes at an earlier age than women, and people living in low-income countries also develop diabetes at an earlier age than those in high-income countries. Furthermore, the age-standardized prevalence of any comorbidity is 33%, and the most frequent comorbidities are hypertension, coronary heart disease, asthma, and CKD (98). Also, these attributes are known to be independently associated with influenza outcomes. For instance, a meta-analysis found that the coexistence of any comorbidity (chronic lung diseases, CVD, malignancy, diabetes, and chronic liver and renal diseases) with seasonal influenza was associated with 53%, 239%, 74%, 139%, and 104% higher odds of pneumonia, hospital admission, ICU admission, and all-cause death, respectively. Moreover, obese people had a higher likelihood of all-cause death, and older people had a 365% and 195% higher likelihood of hospital admission and all-cause death, respectively (39). In a large population-based cohort study in the United Kingdom, the most prevalent comorbidities were obesity (8%), asthma (6%), diabetes (4%), and CVD (3.5%) among patients hospitalized with H1N1. While the presence of chronic pulmonary diseases and obesity significantly increased the likelihood of severe H1N1 by 241% and 596%, respectively, and ICU admission or death by 217% and 508%, respectively (99). Likewise, another meta-analysis demonstrated that older adults with influenza had a risk of hospital admission and the presence of any multimorbidity increased the risk of influenza-related hospitalization by ~2-fold. Regarding severe outcomes, old age and accumulation of comorbidities increased the risk of ICU admission or death following influenza (100). Although the role of clinical characteristics and multimorbidity is thoroughly investigated in both diabetes and influenza, there is a dearth of studies evaluating the impact of these factors on severe influenza infection and its adverse outcomes in people with diabetes.

1.12. COVID-19 and diabetes mellitus

Understanding regarding the relationship between diabetes and COVID-19 has evolved in light of evidence over the course of the pandemic. Initially, diabetes was associated with an increased risk of severe SARS-CoV-2 infection, ICU admission, need for mechanical ventilation, pneumonia, ARDS, end-organ failure, and mortality (101). In later phases of the pandemic, newer evidence emerged suggesting the association of SARS-CoV-2 infection with the incidence of insulin resistance and diabetes (102). In addition, COVID-19 has also been reported to substantially increase the risk of acute complications of diabetes such as DKA and worsen the progression of existing diabetes-related chronic complications (103,104). Like influenza, the reciprocal association between diabetes and COVID-19 has been increasingly recognized in the recent literature, thus creating a challenging scenario for clinicians in tackling these diseases (105).

1.12.1. Adverse COVID-19 outcomes and diabetes mellitus

In the first wave of the COVID-19 pandemic, diabetes emerged as one of the major risk factors for developing severe SARS-CoV-2 infection, measured by different severity scores as well as indicated by hospitalization, ICU admission, and the need for mechanical ventilation. With the availability of more evidence over the course of the pandemic, diabetes has been found to increase the risk of both pulmonary and extra-pulmonary complications such as pneumonia, ARDS, adverse cardiovascular events, worsening of existing comorbid conditions and diabetes-related complications, multiple organ failure, and mortality (18,106).

Epidemiological data show that countries with a high burden of diabetes have reported higher rates of SARS-CoV-2 infection and its related mortality compared to countries with a lower burden of diabetes (18). Also, T2DM has been reported as the second most prevalent comorbidity of COVID-19, with a global pooled prevalence of 17% (107). In addition, a meta-analysis revealed that the pooled mortality related to COVID-19 was significantly higher in people with diabetes (22% vs. 13%, $p < 0.05$) compared to those without it (107). Regarding the severity of COVID-19 and diabetes, an umbrella meta-analysis comprising studies from Europe, the Middle East, the Far East, and America reported that patients with diabetes had 59% higher pooled odds of ICU admission and 44% higher pooled odds of ventilation requirements compared to those without diabetes. In addition, diabetes increased the pooled odds of developing a severe clinical condition by 188% and mortality by 87% (108).

As T2DM represents most cases of diabetes (90–95%) (1), most studies thus far have either assessed only the association of T2DM with COVID-19 or have not explicitly categorized diabetes into its subtypes in their analyses. However, a limited number of studies have explicitly investigated the association of T1DM and T2DM with COVID-19 severity and prognosis; but no differences were observed between the two subtypes despite a vast difference in their pathogenesis and risk factors. In this regard, a countrywide study from Sweden showed that T2DM increased the odds of COVID-19-related hospitalization, ICU admission, and death by 2-fold each. While T1DM increased the odds of death by 3-fold; however, it was not significantly associated with an increased risk of COVID-19 severity (109). Another single-centre study from the United States stated that both T1DM and T2DM shared similar increased odds of hospitalization (T1DM: 3.90, T2DM: 3.36) and disease severity (T1DM: 3.35, T2DM: 3.42) compared to non-diabetes (110). Also, a meta-analysis revealed that patients with T2DM had an insignificantly higher likelihood of mortality compared to those with T1DM (108).

Studies from several countries have reported a noticeable rise in the incidence of DKA in people with T1DM and T2DM during the COVID-19 pandemic. A multi-centre cross-sectional study conducted in England found that the severity of DKA was significantly higher among children and adolescents during the first wave of the COVID-19 pandemic (mild: 6% vs. 13%, moderate: 24% vs. 7%, severe: 47% vs. 10%, $p = 0.002$) compared to pre-pandemic years (111). In Kuwait, a higher proportion of children (<12 years old) with incident T1DM were presented with DKA (52% vs. 38%, $p < 0.001$) and admitted to paediatric ICU (20% vs. 11%, $p = 0.002$) during the COVID-19 pandemic compared to the pre-pandemic year. In addition, the COVID-19 pandemic was associated with 73% higher odds of DKA development and 104% higher odds of ICU admission (112). Another retrospective analysis of children and adolescents from two tertiary care hospitals in Canada showed that the incidence of overall (68% vs. 46%, $p < 0.001$) and severe (27% vs. 13%, $p = 0.010$) DKA among newly diagnosed T1DM was significantly higher during the COVID-19 pandemic compared to the pre-pandemic year (113). Likewise, a countrywide study from England observed that DKA admissions increased by 6% in the first and post-first waves each and 7% in the second wave of the pandemic compared to previous years (103). The findings of all studies suggest that vigilant glucose monitoring and management are required during COVID-19 in both T1DM and T2DM patients to prevent DKA.

1.12.2. Mechanisms responsible for severe COVID-19 and its adverse outcomes in diabetes mellitus

Growing evidence suggests that various interrelated mechanisms and etiological factors may contribute to severe COVID-19 and its adverse outcomes in diabetes.

Hyperglycaemia has been identified to play a vital role in increasing the risk for severe COVID-19 and its poor prognosis in people with diabetes. It facilitates the replication of SARS-CoV-2 and increases the expression of ACE2 in pulmonary monocytes, which induces cytokine storm, inhibits T-cell response, and eventually damages pulmonary epithelial cells (104,114,115). Hyperglycaemia also causes structural and physiological changes in pulmonary epithelial cells, thus, making people with diabetes more vulnerable to complications like pneumonia, ARDS, respiratory failure, and eventually multiple organ damage (104). In addition to hyperglycaemia, insulin resistance and glycosylation of lung proteins in diabetes may also significantly contribute to inducing pulmonary dysfunction by reducing lung volume and compliance and increasing airway resistance (104).

Apart from inducing pathogenic changes in the lungs, hyperglycaemia triggers disturbances in the coagulation cascade, promotes endothelial dysfunction, and increases the production of inflammatory cytokines and subsequent cytokine storm. These dysregulated processes elevate the risk of thrombo-embolic and cardiac events and injury to various organs in people with diabetes (104,114). The contribution of hyperglycaemia to the severity of COVID-19 and its adverse outcomes in diabetes is supported by clinical and epidemiological studies as well. Inadequate glycaemic control has been associated with higher COVID-19-related complication and mortality rates in people with diabetes. While adequate glucose control during COVID-19 has been demonstrated to achieve better viremia control and improve the prognosis of COVID-19 (108,114). This evidence indicates the importance of maintaining optimal glucose levels during COVID-19 infection (115).

Transient hyperglycaemia has been identified as a common complication of SARS-CoV-2 infection, which develops due to cytokine storm and dysregulated immune response. Both transient hyperglycaemia and cytokine storm may also facilitate oxidative stress, lead to insulin resistance, and impair the function of β cells, ultimately developing a vicious cycle of transient and persistent hyperglycaemia (116). Evidence also suggests that acute glycaemic excursions are critical in triggering endothelial dysfunction. As these excursions occur more frequently and last longer in people with diabetes, the risk of endothelial dysfunction increases substantially, which may contribute to developing thrombo-embolic events during COVID-19 in people with diabetes (114,116). Clinical studies have confirmed the occurrence of transient

hyperglycaemia during SARS-CoV-2 infection, and because of it, a significant proportion of patients had to be switched from oral hypoglycaemic drugs to insulin therapy to manage it. Moreover, as mentioned above, the incidence and severity of DKA events have increased in COVID-19 patients partly due to SARS-CoV-2-induced hyperglycaemia (104,114,115).

Another reason for severe COVID-19 in people with diabetes is their compromised innate and adaptive immune systems. They are in a chronic low-grade proinflammatory state with persistently high levels of IL-1, IL-6, IL-8, and TNF-α, which increases the risk of developing cytokine storm during COVID-19. In addition, defects in chemotaxis and phagocytic activity of neutrophils and a decrease in the production of CD4+ and CD8+ T cells contribute to immune system dysfunction in diabetes. Hyperglycaemia again plays a crucial role in weakening the immune system by disrupting the production of cytokines and promoting neutrophil dysfunction (104,114,115). In addition to the compromised host immune system, the SARS-CoV-2 invades CD4+ T and CD8+ T cells and causes cell apoptosis and lymphocytopenia, further weakening the immune system. Hyperglycaemia has also been suggested to directly increase and sustain the proliferation of the SARS-CoV-2 virus in monocytes and inhibits T-cell response. Consequently, the immune system cannot efficiently fight against the SARS-CoV-2 infection, resulting in a prolonged and severe course of the disease and poor prognosis (104,114,115).

It is well known that lactate regulates inflammatory response modulation, innate immunity suppression, and T helper cell differentiation. Research shows that hyperglycaemia significantly upregulates LDH, thereby increasing the production of lactate (104,115). Accumulating evidence also reveals that LDH levels increase significantly in people with diabetes following the SARS-CoV-2 infection. Therefore, elevated levels of glycolysis-induced lactate are speculated to delay the clearance of SARS-CoV-2, leading to a severe or even fatal outcome in people with diabetes (115).

Recent studies reveal that the SARS-CoV-2 virus can directly damage pancreatic β cells via ACE2 receptors, which can then lead to insulin deficiency and hyperglycaemia, ultimately increasing the risk for severe COVID-19 (117). A study that examined the SARS-CoV-2 infected human pancreatic β cells found that the virus selectively infects islet β cells in vitro, reduces pancreatic insulin secretion, and induces β-cell apoptosis (118). Similarly, an autopsy study showed that SARS-CoV-2 directly infected pancreatic β-cells, caused morphological changes in organelles, and significantly reduced insulin-producing granules, thereby reducing insulin secretion (119). In contrast, a recent in-vitro study of human pancreatic cells claimed that the cytopathy caused by the SARS-CoV-2 virus is generalized to

all types of pancreatic cells, and the virus may not be capable of inducing sufficient damage to β cells on its own to cause diabetes (120). Besides direct damage to β cells, severe SARS-CoV-2 infection can result in abnormal activation of immune and proinflammatory responses, which may persist for months and impair insulin sensitivity in muscle, fat, and liver cells. However, existing evidence does not confirm that the SARS-CoV-2 virus induces β-cell dysfunction and apoptosis (104,115,121).

Another recently discovered mechanism for the increased susceptibility and severity of COVID-19 in diabetes is related to ACE2 receptors. These receptors have been found overexpressed in patients with diabetes, suggesting that people with diabetes are more susceptible to contracting the SARS-CoV-2 infection (116). As ACE2 receptors are also abundant in the endothelium of the heart and kidneys; their overexpression in these cells could be one of the reasons for high extrapulmonary complication rates in diabetes. In addition, hyperglycaemia can induce abnormal glycosylation of the ACE2 receptors, promoting the SARS-CoV-2 virus binding to these receptors and increasing the susceptibility and severity of disease in people with diabetes. Interestingly, insulin has been shown to reduce the expression of ACE2 receptors, which explains why well-controlled glucose is associated with lower COVID-19 severity and improved prognosis (104,115).

Glycated haemoglobin has been suggested as an important factor for COVID-19 mortality in people with diabetes. Surface proteins of the SARS-CoV-2 virus can deoxygenate haemoglobin by dissociating iron to form porphyrin in the erythrocytes, which compromises their blood carrying capacity and leads to the development of respiratory distress. As glycated haemoglobin is significantly elevated in people with hyperglycaemia and diabetes, they are more susceptible to SARS-CoV-2 infection and COVID-19-associated mortality due to excessive glycation of haemoglobin (115).

Earlier in the COVID-19 pandemic, underlying mechanisms responsible for developing severe COVID-19 and its adverse outcomes were unclear. In later phases of the pandemic, the availability of more data revealed that apart from diabetes, risk factors such as old age, male gender, obesity, smoking, and various comorbidities such as CVD, dementia, liver disease, CKD, and cancer also contribute significantly to developing severe COVID-19 and its adverse outcomes (122–125). Old age, male gender, and obesity are established risk factors for T2DM, and the abovementioned comorbidities also frequently cluster in people with diabetes (62). Hence, it is plausible that the presence of such risk factors and comorbidities in people with diabetes may exacerbate the risk of developing severe COVID-19 and its associated adverse outcomes (126). A few studies conducted earlier in the pandemic have

accounted for some of these factors and comorbidities while investigating the association between diabetes and adverse COVID-19 outcomes. In this regard, a study reported that the in-hospital mortality rate was significantly greater for patients with diabetes versus non-diabetes patients; however, in the multivariate analysis that adjusted for other risk factors, the association no longer remained significant. Moreover, patients with diabetes were significantly older (76 vs. 63 years, $p < 0.05$), males (71% vs. 29%, $p < 0.05$), and had a higher prevalence of hypertension (84% vs. 50%, $p < 0.05$) and CVD (45% vs. 15%, $p < 0.05$) (127). Similarly, another study demonstrated that the risk of ICU admission and/or in-hospital mortality for COVID-19 was similar in patients with or without diabetes (hazard ratio: 1.16, 95%CI: 0.95–1.41, $p = 0.14$) (128). However, evidence regarding the contribution of various factors in diabetes and COVID-19 outcomes is still premature (129). A meta-analysis has investigated various clinical phenotypes in people with diabetes that could be associated with COVID-19 severity and death. Its results showed that the risk of COVID-19-related mortality was 28% higher in males, 249% higher in those aged >65 years, 56% higher in those with CVD, 93% higher in those with chronic kidney disease, and 40% higher in those with COPD. In addition, the severity of COVID-19 was 36% higher in males, 67% higher in those aged >65 years, 28% higher in those with CVD, and 36% higher in those with COPD. However, the authors strongly recommended more prospective studies investigating the role of clinical phenotypes and comorbidities to improve the quality of existing evidence (130).

1.12.3. COVID-19 and new-onset diabetes mellitus

Accumulating evidence shows that the incidence of diabetes has increased during the COVID-19 pandemic either due to the SARS-CoV-2 infection or an indirect impact of the pandemic. The pooled global incidence of recently diagnosed diabetes during COVID-19 is estimated as 14% (95%CI: 6–26%) (102). Meanwhile, a 25% to 80% rise in the incidence of T1DM has been reported in different countries during COVID-19 (131–133).

Various prospective studies have investigated the association of SARS-CoV-2 infection with the incidence of unspecific diabetes in adults. In a prospective study of three databases in the United States, the risk of incident diabetes following six months after COVID-19 was 2.47 (95%CI: 1.14–5.38) in adults with COVID-19 compared with matched controls (134). While another retrospective cohort study based on the Veterans health database of the United States revealed that the SARS-CoV-2 infection was associated with an increased risk of incident diabetes among men at 120-days (OR: 2.56, 95%CI: 2.32–2.83] and an entire study period (OR: 1.95, 95%CI: 1.80–2.12) compared to healthy controls. Likewise, among hospitalized patients, the SARS-CoV-2 was associated with a higher risk of diabetes at 120

days (OR: 1.42, 95%CI: 1.22–1.65) and for the entire study period (OR: 1.32, 95%CI: 1.16–1.50) in men (135). Also, a recent retrospective cohort study from the Veterans health data of the United States showed that people who developed COVID-19 were 40% (HR: 1.40, 95%CI: 1.36–1.44) more likely to develop T2DM compared to those without any history of COVID-19 during a follow-up of one year. In addition, the risk of developing diabetes increased with increasing severity of COVID-19 as people who were hospitalized or admitted to ICU had a 2-fold higher risk of developing diabetes compared to those without COVID-19 (132). Consistent with the data from the United States, a large nationwide prospective study from Germany with a median follow-up time of 119 days showed that the incidence of diabetes was 15.8 per 1000 in people with COVID-19 compared to 12.3 per 1000 person-years in people with acute upper respiratory tract infections, which indicated that the risk of developing T2DM was 28% higher in people with COVID-19 than those with respiratory infections (133). Similarly, a large cohort study of hospitalized COVID-19 patients (mean follow-up: 140 days) from England reported that the incidence of diabetes was 29 (95%CI: 26–32) per 1000 person-years with a risk ratio of 1.50 (95%CI: 1.40–1.60) in COVID-19 positive cases compared with healthy controls (136). According to a pooled analysis of three large studies, the risk of developing diabetes was 59% higher (HR: 1.59; 95%CI: 1.40–1.81, p <0.001) after COVID-19 infection compared to healthy controls (137).

Studies have also investigated the association of SARS-CoV-2 infection with T1DM specifically. A large retrospective cohort study by the Center for Disease Control assessed and compared the risk of incident diabetes in children and adolescents with and without COVID-19 who were enrolled in IQVIA and HealthVerity databases. In both databases, the SARS-CoV-2 infection was significantly associated with an increased risk of incident T1DM (IQVIA: HR: 2.66, 95%CI: 1.98–3.56 and HealthVerity: HR: 1.31, 95%CI: 1.20–1.44) compared to those without SARS-CoV-2 infection (138). While a study conducted among Finnish children mentioned that the average number of children admitted to the paediatric ICU due to incident T1DM-related DKA increased from 6.25 in 2016–2019 to 20 in 2020 with an incident ratio of 3.24 (95%CI: 1.80–5.83, p <0.001). However, all 33 children with new onset T1DM during COVID-19 were tested negative for SARS-CoV-2 antibodies, suggesting no direct influence of the virus on the incidence of T1DM (139). Likewise, a study conducted in the Canary Islands of Spain observed an insignificant rise of 26% in the incidence of new T1DM during the COVID-19 pandemic compared with the previous ten years. Moreover, of 33 children examined for SARS-CoV-2 antibodies, 32 children were detected negative, indicating no direct association between the SARS-CoV-2 infection and the incidence of T1DM (140). Also, a study from Kuwait reported that the incidence of T1DM per 100,000 did not differ significantly between

the pre-pandemic period (37.6, 95%CI: 33.6–42.1) and during COVID-19 (40.2, 95%CI: 36.0–44.8) (112). While a recent study compared the prevalence of SARS-CoV-2 antibodies among children and adults with and without newly diagnosed T1DM. The prevalence of SARS-CoV-2 antibodies in people with incident T1DM (0.8%, 95%CI: 0.1%–4.2%) was not significantly different from the general population (2.8%, 95%CI: 1.8%–4.6%) (141). According to a Scottish study among people aged under 35 years, the SARS-CoV-2 infection within the last 30 days was associated with 2.62 times (95%CI: 1.81–3.78) increased risk of incident T1DM; however, no significant association (risk ratio: 0.86, 95%CI: 0.62–1.21) was observed between incident T1DM and SARS-CoV-2 infection >30 days (142).

1.12.4. Factors contributing to the association between COVID-19 and new-onset diabetes mellitus

Although epidemiological and clinical evidence strongly suggests that COVID-19 increases the risk of incident diabetes, it is argued that the observed association is unlikely to be causal and instead driven by various factors indirectly related to the COVID-19 pandemic and diabetes.

Like influenza and other viral infections, the SARS-CoV-2 has been suggested to directly damage pancreatic β cells via ACE2 receptors, eventually leading to decreased insulin production and diabetes. Also, studies of pancreatic cells infected with the SARS-CoV-2 virus have found cytopathic changes in pancreatic cells and a reduction in the quantity of insulin-secreting granules and quantity of insulin (117–119). In addition, a recent clinical study has also found higher levels of islet and glutamic acid decarboxylase autoantibodies in people with newly diagnosed diabetes during the COVID-19 pandemic (143). In contrast, a recent study has contested that the pancreatic damage induced by the SARS-COV-2 virus is unspecific to β cells and the cytopathic changes in the pancreatic cells alone are insufficient to cause diabetes (120). Also, the SARS-CoV-2 antibodies were either not detected in people diagnosed with T1DM during the COVID-19 pandemic or were found in similar proportions between T1DM and non-T1DM children (139,140,144). The SARS-CoV-2 can also injure β cells indirectly by triggering proinflammatory cytokines and promoting autoimmunity. Also, the virus can interfere with insulin receptors present in the liver, adipose, and skeletal cells, thus leading to insulin resistance (145). However, it remains controversial whether SARS-CoV-2-induced transient changes in glucose metabolism could persist long enough to cause diabetes (145).

Several other factors have also been thought to increase the incidence of diabetes during the COVID-19 pandemic. It is possible that many patients were either undiagnosed or in a prediabetic state, and alterations in glucose metabolism induced by the SARS-CoV-2 infection had unmasked these cases. Moreover, cortico-steroids are administered to severe COVID-19 patients as a part of the treatment protocol, which might also induce transient hyperglycaemia. However, the probability of a causal association between steroid therapy and diabetes is highly unlikely. Worsening of lifestyle risk factors such as obesity and inadequate physical activity could be another reason for an increased risk of diabetes during the COVID-19 pandemic (138,145).

1.13. Role of vaccination in diabetes mellitus

1.13.1. Overview of COVID-19 vaccines

As COVID-19 contributes to significant morbidity and mortality worldwide, vaccines were produced to fight against this devastating pandemic. To date, several vaccines have been produced and approved for protection and treatment against COVID-19 (146).

Three different approaches have been adopted to develop vaccines for COVID-19. These approaches are classified based on whether they use the whole virus, parts of viral components that can elicit an immune response, or the genetic material of the virus that can generate specific proteins to elicit an immune response (146,147). The whole-virus approach adopts three different methods for developing vaccines. In the first method, the virus is killed or inactivated using chemicals, heat, or radiation to produce inactivated vaccines. Valneva VLA2001, Bharat Biotech, Sinopharm, and Sinovac are examples of inactivated COVID-19 vaccines. In the second method, the virus is attenuated but kept alive so that it can only trigger an immune response without causing disease. An example of such type of COVID-19 vaccine is COVI-VAC. In the third method, the non-virulent part of an adenovirus is used as a platform to deliver specific proteins or genetic material of the disease-causing virus that can trigger an immune response without causing the disease. Examples of viral vector vaccines are Johnson & Johnson's Janssen COVID-19 Vaccine, AstraZeneca, and Sputnik V. The subunit approach uses specific subunits of the virus such as proteins or sugars that mimic the virus and therefore can be recognized by the host immune system to trigger an immune response. The Novavax vaccine is an example of a protein subunit vaccine for COVID-19. The newer cutting-edge genetic approach uses a genetically engineered genetic material such as DNA or messenger RNA (mRNA) that delivers instructions for synthesizing specific proteins but not the whole

virus. These specific proteins can be recognized by the host immune system to produce an immune response without causing the disease. Moderna and BioNTech/Pfizer are mRNA-based vaccines (146–149).

1.13.2. Immunogenicity of COVID-19 vaccination

Research suggests that among four structural proteins (S, E, M, and N) of the SARS-CoV-2 virus, the S-protein plays a crucial part in eliciting both cellular and humoral immune responses in humans. The S-protein, particularly the RBD antigen has been shown to trigger neutralizing antibodies (NAbs) and T-cell immune responses. The SARS-CoV-2 RBD-specific IgG has been found to contribute to half of the S-protein-induced antibody responses (148). In addition, RBD-specific antibodies and T cells have been detected in COVID-19 patients. Also, NAbs titres have demonstrated a significant correlation with the anti-RBD IgG. More evidence indicates that immunization with RBD resulted in inducing NAbs in rodents. Given this confirmatory evidence, the SARS-CoV-2 S-protein has become a key target for COVID-19 vaccines to elicit a potent immune response and confer subsequent protection against the infection. Other proteins such as N, M, and nsps could also serve as antigens for the host immune system. Furthermore, interactions of viral proteins with host factors have been associated with low interferon-I and interferon-III and elevated proinflammatory cytokine levels. Some nsps, N, M, and other accessory proteins have also been shown to interact with various immune processes. In addition, the interaction between NAbs and the activation of T cells has been reported. While recent evidence suggests a correlation of S, M, nsps, and N proteins with CD4+ and CD8+ T cells (148).

Research shows that COVID-19 vaccines trigger an adequate immune response that offers sufficient protection against the active SARS-CoV-2 virus. According to a meta-analysis of 25 randomized clinical trials, among different types of vaccines, mRNA-based and adenovirus vector-based vaccines demonstrated excellent efficacy against RBD, S-protein, and NAbs after first and second doses (150). Another meta-analysis revealed that the pooled mean efficacy and effectiveness was 76% for COVID-19 vaccines but varied with respect to vaccine types: 65% for BNT162b2, 82% for mRNA-1273, and 69% for Ad26.COV2 (151). Global data confirm that COVID-19 vaccination has played a fundamental role in controlling the COVID-19 pandemic. It is estimated that COVID-19 vaccination has averted approximately 14.4 million COVID-19-related deaths in 185 countries between December 2020 and December 2021, representing a 63% reduction in total deaths globally. Moreover, the COVID-19 vaccination has averted 41% of excess mortality thus far, which is a remarkable achievement in such a short time (152).

1.13.3. Immunogenicity of COVID-19 vaccination in diabetes mellitus

As diabetes substantially increases the risk of COVID-19 severity and poor prognosis – people with diabetes are globally prioritized for treatment and preventive measures, including national immunization programs against COVID-19 (153).

Vaccines function by eliciting an immune response that mimics the infection but without causing serious clinical illness. Therefore, eliciting an optimal immune response is necessary to reap the desired benefit from vaccination. As mentioned previously, COVID-19 vaccines have achieved this goal successfully in the general population (151). However, it remains to be investigated whether COVID-19 vaccines can achieve the same target in the high-risk population of diabetes.

Several reasons pose concerns over the immunogenicity of COVID-19 vaccination in the diabetes population. Both innate and adaptive immune systems are compromised in people with diabetes, which is one of the primary reasons they experience severe COVID-19 and poor prognosis (104). Interestingly, an adequate humoral immune response against the SARS-CoV-2 infection has been reported among people with diabetes previously. In contrast, a significant dysregulation in the cellular immune response, particularly in T cells and cytokines, has been observed during the SARS-CoV-2 infection. While both lymphocyte count and lymphopenia incidence were found to be similar between people with and without T2DM (154). Conversely, various sub-populations of T cells and natural killer cells have been shown to decrease during the SARS-CoV-2 infection in people with T2DM compared to non-T2DM (155). These conflicting research findings raise a concern that people with diabetes, similar to the immunogenic response to SARS-CoV-2 infection, may elicit a reduced or at least dysregulated immune response to COVID-19 vaccination as well, which can subsequently hamper the efficacy and effectiveness of vaccination in this population.

The concern regarding the suboptimal immunogenicity of COVID-19 vaccination in people with diabetes also stems from the previous research on hepatitis B, varicella zoster, and influenza vaccination. As such, the hepatitis B vaccination induced a lower median sero-protection in 73% of people with diabetes compared to 87% in those without diabetes (156). While the influenza vaccination offered similar protection and effectiveness in people with and without diabetes (157). Likewise, evidence on the immunogenicity of varicella zoster and pneumococcal vaccines in diabetes is inconclusive – hence warranting the need for more robust studies on the immunogenicity of vaccines in this population (158). Clinical studies comparing the immunogenicity of COVID-19 vaccination in diabetes versus non-diabetes populations are sparse. To our knowledge, one study has compared both cellular and humoral

immunogenic responses to COVID-19 vaccination in well-controlled and insufficiently controlled people with diabetes and healthy controls. This study demonstrated substantially lower immune response in people with diabetes than those without diabetes (159). While another study found lower levels of IgG RBD levels and NAbs antibody titres in people with diabetes (0.88, 95%CI: 0.79–0.98). However, the number of people with diabetes was small to achieve valid estimates (160). Additionally, phase III clinical trials of mRNA and adenovirus-based COVID-19 vaccines have compared their efficacy rates between diabetes and non-diabetes. However, detailed analyses of specific clinical phenotypes and characteristics of vaccine recipients are not reported (161–165). As clinical characteristics can vary substantially within diabetes and among its subtypes and can also be significantly associated with the immunogenicity of COVID-19 vaccines, it is imperative to account for such characteristics to elucidate the independent impact of diabetes on the immune response.

Diabetes-induced chronic hyperglycaemia is another crucial and independent factor contributing to the low immunogenicity of vaccines. As previously discussed, hyperglycaemia can disrupt both innate and adaptive immune responses by causing lymphocytopenia, impairing monocyte, macrophage, and neutrophil function, and dysregulating complement activation dysfunction (104). Therefore, it can directly or indirectly compromise the activation of a desired immune response to vaccinations, including COVID-19 (156). Some clinical studies have demonstrated a significant negative impact of poorly controlled diabetes, measured by the HbA1c, on the prognosis of COVID-19 (166,167). However, the HbA1c was an insignificant predictor of mortality in COVID-19 patients in our previous study (62). Interestingly, an earlier study identified poorly controlled glycaemia (HbA1c) as the main culprit for the reduced immunogenic response to COVID-19 vaccination in people with diabetes (159). However, more research is necessary to establish a clear impact of dysglycaemia on the immunogenicity of COVID-19 vaccination.

1.13.4. Impact of COVID-19 vaccination on glycemia and diabetes management

As mentioned earlier, transient hyperglycaemia is a commonly reported complication of SARS-CoV-2 infection that develops due to insulin resistance in peripheral cells secondary to virus-induced cytokine storm, release of other proinflammatory markers, endothelial dysfunction, and a direct insult to the pancreas (104,115). These transient glycaemic changes may increase daily insulin dosing requirements or demand an urgent adjustment in antidiabetic medicines. These glycaemic changes, if not managed appropriately, can lead to serious complications like DKA – which is evident from a significant rise in DKA events during the COVID-19 pandemic (103,112). People with diabetes, which comprise a significant proportion

of the population in any country, are being vaccinated against COVID-19 on a priority basis. However, serious reservations exist among this population regarding vaccine-triggered hyperglycaemia and its impact on diabetes management. As vaccination and natural infection elicit the immune response in a similar pattern, the vaccine-induced immune response may also trigger transient hyperglycaemia. This vaccine-induced hyperglycaemia, like the SARS-CoV-2 infection, may need to be managed by adjusting insulin doses or other antidiabetic medicines.

Earlier studies have clearly shown the benefits of adjusting insulin dosage and strict glycaemic control for achieving a favorable prognosis of SARS-CoV-2 infection (108,114). However, only two case studies have described the cases of hyperglycaemia after receiving the first COVID-19 vaccination, which resolved on its own in most instances without any additional intervention or adjusting the medication (168,169). However, the frequency of hyperglycaemia after COVID-19 vaccination is yet unknown. In addition, the association between these two occurrences and their impact on the management of diabetes and carbohydrate intake are yet to be explicitly elucidated in people with T1DM and T2DM.

2. AIMS AND OBJECTIVES

Diabetes has been shown to substantially elevate the risk of developing severe influenza infection and its associated complications. Despite this incriminating evidence, people with diabetes represent a heterogenous population that is characterized by a broad age range, various subtypes of diabetes, and a varying burden and severity of concurrent morbidities. As these characteristics are also established risk factors for adverse influenza outcomes, it is reasonable to assume that these characteristics may individually or cumulatively increase the risk of hospitalization and fatal influenza in people with diabetes. However, their influence on influenza outcomes is not well understood and widely investigated in large diabetes cohorts. In addition, influenza-associated hospitalization and mortality rates have been shown to increase significantly in recent years, which warrants the need to identify specific reasons behind this trend. **In light of these research needs, this study aimed to evaluate the impact of characteristics and comorbidities on both hospitalization and short-term mortality rates following hospitalization for influenza-related illness (IRI) in people with diabetes.** As many countries have been witnessing a substantial rise in seasonal influenza epidemics in recent years, it is imperative to identify high-risk influenza groups within the diabetes population to effectively organize and implement surveillance and vaccination programs and allocate healthcare resources efficiently.

Like influenza, a vast majority of research conducted in the early phase of the COVID-19 pandemic implicated diabetes as a major risk factor for severe COVID-19 infection and its worse prognosis. With the availability of more clinical and epidemiological data over the course of the pandemic; sex, age, obesity, smoking status, and the presence of certain chronic diseases such as CVDs, COPD, CKD, chronic liver disease, and cancer also emerged as significant risk factors of adverse outcomes of COVID-19. As people with diabetes also share most of these risk factors and frequently suffer from multimorbidity, therefore, we hypothesized that these underlying risk factors and specific comorbidities might be responsible for the worse prognosis of COVID-19 in people with diabetes rather than diabetes alone. However, research disentangling the contribution of these underlying factors in developing adverse outcomes in COVID-19 patients with diabetes is still emerging. **Therefore, this study investigated the impact of diabetes on the severity of COVID-19 infection and associated in-hospital mortality independent of the factors mentioned-above by utilizing the propensity-score matching technique.**

Culminating evidence suggests that COVID-19 induces detrimental effects in multiple organ systems via various complex pathophysiological mechanisms related to myocardial stress, hypoxia, coagulation, and inflammation, among others. These deleterious effects of COVID-19 may alter various inflammatory, coagulation, hepatic, and cardiac biomarkers and may provide valuable information regarding its severity and prognosis. Although, many studies have identified several biomarkers of COVID-19 severity and mortality, people with diabetes have a weak immune system and dysfunctional inflammatory response and are more prone to cardiac injury. Therefore, it can be argued that alterations in certain inflammatory, coagulation, and cardiac biomarkers may be more pronounced in people with diabetes. However, most studies have limited their analyses to determine the associations of selective biomarkers with COVID-19 outcomes without measuring their predictive performance. In addition, there is a dearth of studies investigating the predictive role of these biomarkers in COVID-19 patients with diabetes. **Therefore, we assessed the performance of various inflammatory, hepatic, coagulation, and cardiac biomarkers to predict mortality in patients with prediabetes and diabetes hospitalized for COVID-19.**

The SAPS 3 is administered as a part of routine care in critical care units in Austria to evaluate the prognosis of severely ill patients and assist in their clinical management. As people suffering from COVID-19 frequently develop serious complications that often require hospitalization and admission to critical care units – the SAPS 3 is also being utilized in these patients in the country. We postulated that SAPS 3 might demonstrate an unsatisfactory or a varying degree of predictive performance in COVID-19 patients because it predicts the prognosis of patients based on specific clinical parameters and biomarkers that may not fully capture the pathophysiological alterations induced by COVID-19. Some countries have evaluated the performance of SAPS 3 in COVID-19 patients; however, findings regarding its prognostic performance were conflicting. Also, issues were reported regarding the ability of this tool to classify patients accurately into various risk categories, which questions its clinical utility in COVID-19 patients. Besides the endogenous issues in this tool, exogenous factors related to healthcare infrastructure, ICU practice, and treatment protocols may significantly influence the prognosis of COVID-19 patients. Therefore, validating the SAPS 3 tool in the country-specific cohorts is crucial before incorporating it into routine clinical practice. In addition, no study has assessed the performance of SAPS 3 in COVID-19 patients with diabetes thus far, which is recognized as one of the major risk groups for COVID-19 mortality. Given the disagreement in the literature and the abovementioned endogenous and exogenous factors that may influence the performance of this tool, more validation studies are required in large patient cohorts. **Hence, this study evaluated the performance of SAPS 3 to predict**

in-hospital mortality in a countrywide cohort of critically ill COVID-19 patients in Austria. In addition, the predictive performance of SAPS 3 was compared between COVID-19 patients with and without diabetes.

People with diabetes are prioritized globally for vaccination against COVID-19 due to their compromised immune system and high susceptibility to developing adverse outcomes. There is also a concern that COVID-19 vaccination may not elicit optimal immune response in people with diabetes, which may have substantial effects on the efficacy and administration protocol of this vaccination. This assumption is supported by the previous study on the hepatitis B vaccine, which reported a reduced immune response in people with diabetes. While findings regarding the comparison of influenza, varicella, and pneumococcus vaccines between diabetes and non-diabetes were indecisive. Moreover, phase III studies have demonstrated similar efficacy of mRNA- and adenovirus-based COVID-19 vaccines between diabetes and non-diabetes. While a recent study has shown insufficient antibody levels after COVID-19 vaccination in people with diabetes. However, in that study, the number of people with diabetes was small, they were classified into subtypes of diabetes, and the impact of glycaemic control on immune response was not investigated. **To address these research gaps, this study was carried out to 1) elucidate the impact of types of diabetes and glycaemic control on antibody response against SARS-CoV-2 S-protein following first and second doses of COVID-19 vaccine, respectively; 2) investigate and compare the frequency of side effects between T1DM and T2DM after receiving COVID-19 vaccines; and 3) compare anti-SARS-CoV-2 S antibody levels of people with diabetes to healthy controls.**

The vaccine-triggered immune response can increase insulin requirements by altering insulin sensitivity due to the activation of inflammatory, cellular, and humoral immune responses. Because of these vaccine-induced pathophysiological alterations, blood glucose and thereafter diabetes management may be deteriorated after the vaccination, thereby increasing the risk of acute complications. This can be of major concern for vaccine acceptance in people with diabetes. **In the purview of this assumption, this study aimed to investigate 1) the short-term effects of COVID-19 vaccination on glycemia in people with T1DM and T2DM; 2) whether the presence and severity of typical side effects and elevated body temperature following COVID-19 vaccination alter glycemia in people with T1DM and T2DM; and 3) whether COVID-19 vaccination influenced bolus insulin dosing behavior and carbohydrate intake in people with T1DM and T2DM.**

3. MATERIALS AND METHODS

This section summarizes methods of the following six published articles:

1. Aziz F, Aberer F, Moser O, Sourij C, von Lewinski D, Kaser S, Reichardt B, Sourij H. Impact of comorbidities on mortality in hospitalized influenza patients with diabetes - Analysis of the Austrian Health Insurance. *Diabetes Research and Clinical Practice.* 2021;174:108758. doi: 10.1016/j.diabres.2021.108758.
2. Aziz F, Aberer F, Bräuer A, Ciardi C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. COVID-19 In-Hospital Mortality in People with Diabetes Is Driven by Comorbidities and Age-Propensity Score-Matched Analysis of Austrian National Public Health Institute Data. *Viruses*. 2021;30;13(12):2401. doi: 10.3390/v13122401.
3. Aziz F, Stöcher H, Bräuer A, Ciardi C, Sourij H et al., for the COVID-19 in Diabetes in Austria Study Group. Biomarkers Predictive for In-Hospital Mortality in Patients with Diabetes Mellitus and Prediabetes Hospitalized for COVID-19 in Austria: An Analysis of COVID-19 in Diabetes Registry. *Viruses*. 2022;14(6):1285. doi: 10.3390/v14061285.
4. Aziz F, Reisinger AC, Aberer F, Sourij C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. Simplified Acute Physiology Score 3 Performance in Austrian COVID-19 Patients Admitted to Intensive Care Units with and without Diabetes. *Viruses*. 2022;14(4):777. doi: 10.3390/v14040777.
5. Sourij C[#], Tripolt NJ[#], Aziz F[#], Aberer F, Sourij H et al. Humoral immune response to COVID-19 vaccination in diabetes is age-dependent but independent of type of diabetes and glycaemic control: The prospective COVAC-DM cohort study. *Diabetes, Obesity and Metabolism*. 2022;24(5):849–58. doi: 10.1111/dom.14643.
6. Aberer F[#], Moser O[#], Aziz F[#], Sourij C, Sourij H et al., for the COVAC-DM Study Group. Impact of COVID-19 Vaccination on Glycemia in Individuals with Type 1 and Type 2 Diabetes: Substudy of the COVAC-DM Study. *Diabetes Care*. 2022;45(2):e24–6. doi: 10.2337/dc21-1563.

The detailed description of methods for each study can be read in the published articles provided in the appendix 1–6.

3.1. In-hospital mortality in influenza patients with diabetes mellitus

A retrospective cohort study was conducted on 507,184 individuals with T1DM and T2DM enrolled in the countrywide '*Austrian health insurance (AHI)*' database between 2013 and 2017.

Study variables included demographic characteristics, International Classification of Disease (ICD) codes of primary and secondary diseases, discharge date from the hospital, Anatomical Therapeutic Classification (ATC) codes of medicines, MEL codes of medical and surgical procedures, and all-cause mortality along with the date of death.

The diagnoses of T1DM and T2DM were confirmed from the ICD-10 codes and ATC codes of glucose-lowering medicines. Information about primary and secondary episodes of influenza-related hospitalizations was extracted from the ICD-10 codes J09, J10, and J11. As other flu-like illnesses could have been included in these codes, therefore, influenza was defined as an influenza-like illness (IRI). All-cause mortality was defined as death occurring within 30 and 90 days of discharge from the hospital, respectively. A weighted summary score called age-adjusted Charlson Comorbidity Index (ACCI) was generated from the ICD-10 codes of comorbidities: myocardial infarction, congestive heart failure (CHF), peripheral vascular disease, stroke, dementia, COPD, rheumatoid arthritis, peptic ulcer disease, liver disease, diabetes, hemiplegia, chronic kidney disease (CKD), cancer, and acquired immune deficiency syndrome (AIDS) (170). The study was approved by the ethics committee of the Medical University of Graz, Austria.

The association of IRI hospitalization with characteristics, individual comorbidities, and the ACCI score was ascertained with the logistic regression analysis. Cumulative 30- and 90-day mortality rates following IRI were estimated and compared with characteristics, individual comorbidities, and the ACCI score. In addition, multilevel parametric survival regression analyses were performed to assess the associations of cumulative mortality rates with characteristics, individual comorbidities, and the ACCI score.

3.2. Adverse outcomes of COVID-19 in hospitalized patients with diabetes mellitus

A retrospective cohort study was conducted on 40,602 patients hospitalized for primary and secondary SARS-CoV-2 infection in Austria between March 2020 and March 2021 and enrolled in the '*national COVID-19 database (GÖG) of the Austrian National Public Health*

Institute'. The database captured information about age deciles, sex, number of hospitalizations for COVID-19, geographic regions, diabetes, comorbidities, ICU admission, and in-hospital mortality.

The severity of COVID-19 was defined as admission to the ICU following hospitalization for COVID-19. In-hospital mortality was defined as death occurring in the hospital following hospitalization for COVID-19. Diabetes was defined as per ICD-10 codes of E10, E11, E12, E13, and E14. Comorbidities were identified as per ICD-10 codes specified in Charlson and Elixhauser indices. These comorbidities included myocardial infarction, cardiac arrhythmias, valvular heart disease, hypertension, congestive heart failure (CHF), peripheral vascular disease, stroke, chronic obstructive pulmonary disease (COPD), pulmonary circulation disorders, dementia, rheumatoid disease, peptic ulcer disease, liver disease, paralysis, other neurological disorders, CKD, cancer with/without metastasis, Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS), hypothyroidism, coagulopathy, fluid and electrolyte disorders, blood loss anemia, deficiency anemia, alcohol abuse, psychosis, and depression (170,171). The weighted summary score called Charlson Comorbidity Index (CCI) was calculated to estimate the cumulative impact of comorbidities (except diabetes) on outcomes. This study was approved by the ethics committee of the medical university of Graz, Austria.

The association of diabetes with ICU admission and in-hospital mortality was assessed by performing unmatched and propensity-score matched (PSM) logistic regression. In unmatched analysis, the unadjusted association of diabetes with both outcomes was assessed in the entire cohort (N = 40,602). In the PSM analysis, the PSM cohorts of diabetes (n = 4971) and non-diabetes (n = 4971) patients were generated to balance the distribution of characteristics and comorbidities between these cohorts. This statistical method allowed us to estimate the independent association of diabetes with both outcomes.

3.3. Biomarkers of in-hospital mortality in COVID-19 patients with diabetes mellitus

A cohort of hospitalized COVID-19 patients with prediabetes and diabetes was analyzed from the '*COVID-19 in diabetes registry*'. Data for this registry were collected by designated healthcare professionals from medical files and routinely measured laboratory parameters.

The primary outcome, in-hospital mortality, was defined as death from the date of admission till the date of discharge from the hospital. The predictor variables, collected upon hospital admission, included clinical data, comorbidities, COVID-19 and concomitant medicines, and laboratory parameters. Clinical data comprised age, gender, body mass index (BMI), oxygen saturation, vital signs, smoking status, duration of diabetes, length of hospital stay, and requirement for ICU and assisted ventilation. Routinely collected renal biomarkers included eGFR and LDH; hepatic biomarkers included AST, ALT, and AST–ALT ratio; inflammation biomarkers included CRP, ferritin, PCT, and IL-6; coagulation biomarkers included fibrinogen and D-dimer; and cardiac biomarkers included NT-proBNP and Troponin T. The study was approved on 15 April 2020 by the Ethics Committee of the Medical University of Graz, Graz, Austria.

All biomarkers were log-transformed because they were positively skewed. The association of each biomarker was ascertained with in-hospital mortality using simple and multiple logistic regression analyses (adjusted for age, sex, and type of diabetes). The predictive performance of each biomarker was evaluated in terms of discrimination and calibration. Discrimination was assessed by C-statistic or area under the receiver operator characteristic curve (AUC). Calibration was assessed by performing the Hosmer–Lemeshow goodness-of-fit test.

3.4. Performance of SAPS 3 in critically ill COVID-19 patients with and without diabetes mellitus

A retrospective cohort study was conducted on 5,850 adult patients with and without diabetes who were admitted to ICUs following primary or secondary diagnosis of SARS-CoV-2 infection between March 2020 to March 2021 in Austria and enrolled in the '*GÖG*' database. The database collected information on age deciles, sex, diabetes, comorbidities, laboratory parameters, and in-hospital mortality.

In-hospital mortality was defined as death occurring in the hospital following hospitalization for primary or secondary SARS-CoV-2 infection. Diabetes was diagnosed as per ICD-10 codes (E10, E11, E12, E13, E14) and SAPS 3 diagnostic criteria. The SAPS 3 score consists of 20 variables that were recorded at the time of ICU admission. These variables were classified as patient characteristics, reasons for ICU admission, and acute physiological disruptions. Patient characteristics included age deciles (20–90+ years), gender, previous health status, comorbidities, intra-hospital location before ICU, length of stay in the hospital

before ICU admission, and major therapeutic interventions before ICU admission. Reasons for ICU admission included health conditions, status and site of surgery, and the presence of infection at ICU admission. Acute physiological disruptions were measured in terms of vital signs, neurological status, serum creatinine, leukocytes, platelets, blood pH, partial pressure of oxygen (PaO_2), and a fraction of inspired oxygen (FiO_2). This study was approved by the Ethics Committee of the Medical University of Graz, Austria.

The SAPS 3 score was calculated from the variables and recommended algorithm provided in the original publication. The predicted in-hospital mortality was estimated from the SAPS 3 score using the following three versions of logit regression equations (70):

1. Standard equation (global equation)

   Logit = -32.6659 + ln [SAPS 3 score +20.5958] × 7.3068

2. Central European equation

   Logit = -36.0877 + ln [SAPS 3 score + 22.2655] × 7.9867

3. Austrian equation recalibrated for COVID-19 patients:

   Logit = -14.451 + ln [SAPS 3 score + -12.092] × 3.666

The standardized mortality ratio (SMR) was estimated for each SAPS 3 equation. The predictive performance of SAPS 3 equations was assessed via discrimination, calibration, and accuracy. Discrimination was assessed by calculating the area under the receiver operating characteristics curve (AUC) and was compared between patients with and without diabetes using the DeLong test. Optimal cut-off values of SAPS 3 score for the overall, diabetes, and non-diabetes cohorts were identified via the Youden index method. Afterward, sensitivity, specificity, and predictive values of the SAPS 3 score were calculated from these optimal values. Calibration was assessed via the Hosmer–Lemeshow (H-L) goodness-of-fit test and the calibration plot. Accuracy of the SAPS 3 for predicting in-hospital mortality was assessed using the Brier score.

3.5. Humoral immune response to COVID-19 vaccination in people with diabetes mellitus

The '*Immune response to Covid-19 vaccination in people with Diabetes Mellitus (COVAC-DM)*' study was a pragmatic multicentre prospective cohort study, which was conducted at the medical university of Graz, the medical university of Innsbruck, and the

university of Bayreuth, Germany. The current analyses focus on measuring and comparing the immune response after the first and the second dose of the COVID-19 vaccine.

A total of 161 people with established T1DM or T2DM and 86 healthy controls aged 18 to 80 years were recruited from the outpatient clinics of participating centres. The participants received COVID-19 as a part of the Austrian and German national vaccination strategy. People with active malignancy, pregnancy, acute inflammatory disease, alcohol abuse, history of COVID-19 infection, any contraindication to the vaccine, and receiving immunosuppressant therapy were excluded from the study. To investigate the impact of glycaemic control on immune response, study participants were further classified into the following four groups: T1DM with an HbA1c level ≤58 mmol/mol (≤7.5%), T1DM with an HbA1c level >58 mmol/mol (>7.5%), T2DM with an HbA1c level ≤58 mmol/mol (≤7.5%), and T2DM with an HbA1c level >58 mmol/mol (>7.5%).

Study visits were planned 60 to 2 days before the first dose, 7 to 14 days after the first dose, and 14 to 21 days after the second dose of the COVID-19 vaccine. At the baseline visit, information about the medical history and medication was recorded. At each follow-up visit, information about the side and adverse effects of the vaccine was recorded, physical examinations were performed, and blood samples were drawn to measure anti-SARS-CoV-2 S antibody levels. In antibody testing, a CE-marked serological test was performed to determine and quantify specific antibodies against SARS-CoV-2 infection. Total immunoglobulin (Ig) was determined using the Roche Elecsys anti-SARS-CoV-2 S electrochemiluminescence immunoassay targeting the RBD of the viral spike protein. The study was approved by the ethics committees of the Medical University of Graz and the Bayerische Landesaerztekammer and registered at the European Union Drug Regulation Authorities Clinical Trials registry.

The median Anti-SARS-CoV-2 S antibody levels were compared between four diabetes groups, T1DM and T2DM, and healthy controls using the Kruskal-Wallis test. The results were adjusted for age and sex using the quantile regression and for multiple comparisons using the Bonferroni method. In participants with diabetes, correlations between clinical characteristics and anti-SARS-CoV-2 S antibody levels after the second vaccination were assessed with the Pearson correlation test. Side effects of the vaccine were compared between people with T1DM and T2DM using Chi-square or Fisher's exact tests, as appropriate.

3.6. Impact of COVID-19 vaccination on glycemia, insulin dosing pattern, and carbohydrate intake in people with diabetes mellitus

This sub-study of '*COVAC-DM'* included people with T1DM and T2DM who were using continuous glucose monitoring (CGM) system as a part of routine diabetes care around the first COVID-19 vaccination period (2 days before and 3 days after the vaccination). The CGM devices used by participants were Abbott freestyle libre, Dexcom G6, and Medtronic.

The impact of the first dose of the COVID-19 vaccine on glycaemia was measured as time spent in prespecified glycaemic ranges: time below range (TBR: <70 mg/dl), time in range (TIR: 70-180 mg/dL), and time above range (TAR: >180 mg/dL). Information about bolus insulin doses and carbohydrate intake was gathered from participants via a questionnaire during the entire study period. Information about the side effects of the vaccine was recorded for each of the 3 days after the vaccination. In addition, a simple score was created to summarize the presence and severity of vaccine-related side effects for each day. The study was approved by the ethics committees of the Medical University of Graz and the Bayerische Landesaerztekammer.

All CGM datapoints were downloaded from the devices and summarized as mean ± standard deviation. Changes in glycaemia, bolus insulin, and carbohydrate intake over days were assessed separately for people with T1DM and T1DM using the repeated measure analysis of variance (ANOVA) test. The relationship of each glycemic category (TIR, TAR, TBR) was compared with the side effects score using Kruskal Wallis or Wilcoxon rank-sum tests.

4. RESULTS

This section summarizes main results of the following six published articles:

1. Aziz F, Aberer F, Moser O, Sourij C, von Lewinski D, Kaser S, Reichardt B, Sourij H. Impact of comorbidities on mortality in hospitalized influenza patients with diabetes - Analysis of the Austrian Health Insurance. *Diabetes Research and Clinical Practice.* 2021;174:108758. doi: 10.1016/j.diabres.2021.108758.
2. Aziz F, Aberer F, Bräuer A, Ciardi C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. COVID-19 In-Hospital Mortality in People with Diabetes Is Driven by Comorbidities and Age-Propensity Score-Matched Analysis of Austrian National Public Health Institute Data. *Viruses*. 2021;30;13(12):2401. doi: 10.3390/v13122401.
3. Aziz F, Stöcher H, Bräuer A, Ciardi C, Sourij H et al., for the COVID-19 in Diabetes in Austria Study Group. Biomarkers Predictive for In-Hospital Mortality in Patients with Diabetes Mellitus and Prediabetes Hospitalized for COVID-19 in Austria: An Analysis of COVID-19 in Diabetes Registry. *Viruses*. 2022;14(6):1285. doi: 10.3390/v14061285.
4. Aziz F, Reisinger AC, Aberer F, Sourij C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. Simplified Acute Physiology Score 3 Performance in Austrian COVID-19 Patients Admitted to Intensive Care Units with and without Diabetes. *Viruses*. 2022;14(4):777. doi: 10.3390/v14040777.
5. Sourij C[#], Tripolt NJ[#], Aziz F[#], Aberer F, Sourij H et al. Humoral immune response to COVID-19 vaccination in diabetes is age-dependent but independent of type of diabetes and glycaemic control: The prospective COVAC-DM cohort study. *Diabetes, Obesity and Metabolism*. 2022;24(5):849–58. doi: 10.1111/dom.14643.
6. Aberer F[#], Moser O[#], Aziz F[#], Sourij C, Sourij H et al., for the COVAC-DM Study Group. Impact of COVID-19 Vaccination on Glycemia in Individuals with Type 1 and Type 2 Diabetes: Substudy of the COVAC-DM Study. *Diabetes Care*. 2022;45(2):e24–6. doi: 10.2337/dc21-1563.

The detailed description of results for each study can be read in the published articles provided in the appendix 1–6.

4.1. In-hospital mortality in influenza patients with diabetes mellitus

A total of 1,994 people and 2,014 cases were hospitalized due to IRI from 2013 to 2017 at various hospitals across Austria. The number of hospitalizations for IRI peaked from November to February, rapidly declined from March to April, and was the lowest from May to October. Of the individuals with IRI, 50% were coded as J10, 29% as J11, and 21% as J09 (172). See figure 4.1.1 for details.

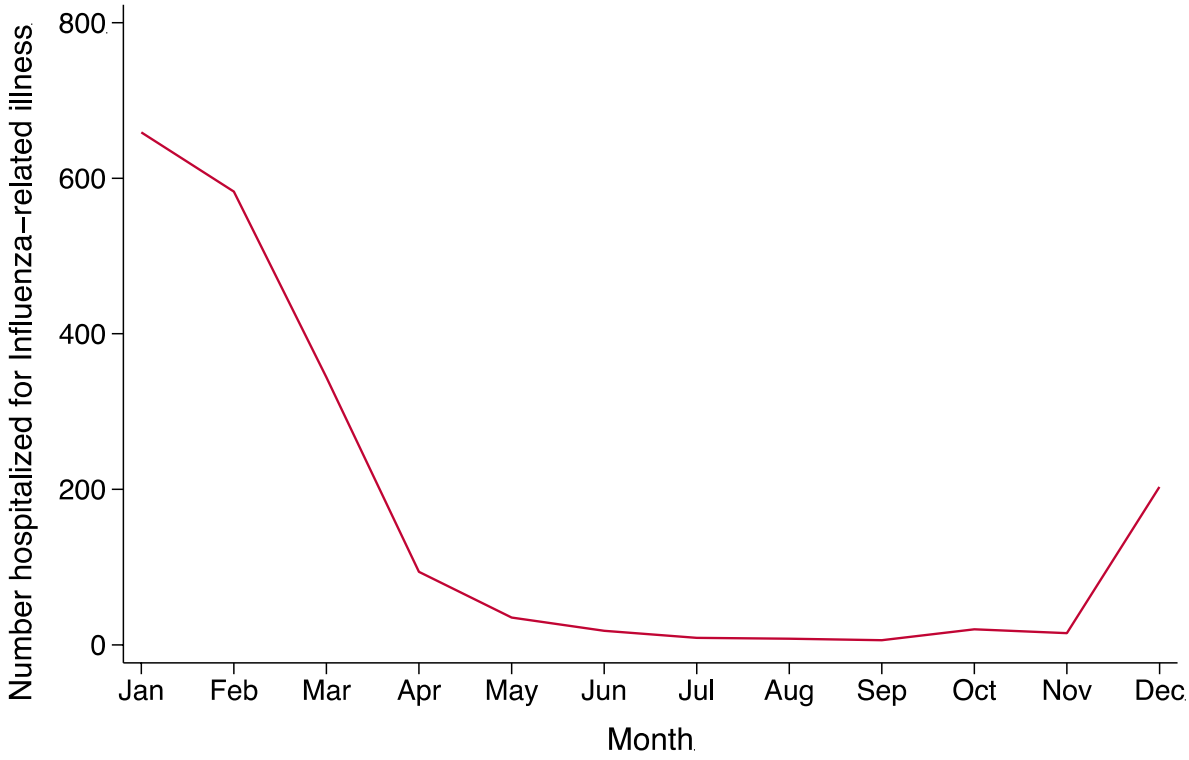

Figure 4.1.1. Seasonal distribution of patients hospitalized for Influenza-related illness in Austria between 2013 and 2017 (N = 1994) (172).

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The comparison between patients hospitalized due to IRI (N = 1994) and other causes (N = 505,190) revealed that the IRI cohort was significantly older (p <0.001) and had a higher burden of comorbidities such as cancer, COPD, CHF, dementia, liver disease, and renal disease compared with the non-IRI cohort. People with T1DM had 33% higher odds of being hospitalized due to IRI compared to those with T2DM. Among comorbidities, individuals with COPD followed by renal disease had 185% and 112% higher odds of hospitalization due to IRI. Each point-score accumulation of the cumulative comorbidity score increased the odds of IRI hospitalization by 25% (172). See table 4.1.1 for details.

Table 4.1.1. Comparison of characteristics between individuals hospitalized for influenza-related illness and other causes (172).

Variables	With IRI (N = 1994)	Without IRI (N = 505,190)	Simple regression		Multiple Regression	
	n (%)	n (%)	OR (95%CI)	P-value	AOR (95%CI)	P-value
Model I						
Age categories						
<50 years	89 (4.5)	47,994 (9.5)	Reference		Reference	
50–59 years	154 (7.7)	67,695 (13.4)	1.23 (0.95–1.59)	0.123	1.14 (0.88–1.49)	0.318
60–69 years	293 (14.7)	115,032 (22.8)	1.37 (1.08–1.74)	0.009	1.14 (0.90–1.45)	0.286
70–79 years	654 (14.7)	143,777 (28.5)	2.45 (1.96–3.06)	<0.001	1.74 (1.39–2.19)	<0.001
80+ years	804 (32.8)	130,844 (25.9)	3.31 (2.66–4.12)	<0.001	2.01 (1.60–2.52)	<0.001
Sex						
Male	1085 (54.4)	255,062 (51.2)	Reference		Reference	
Female	909 (45.6)	243,128 (48.8)	0.89 (0.81–0.98)	0.014	0.85 (0.78–0.93)	0.001
Type of diabetes						
T2DM	1858 (93.2)	485,683 (96.1)	Reference		Reference	
T1DM	136 (6.8)	19,507 (3.9)	1.74 (1.45–2.09)	<0.001	1.33 (1.11–1.59)	0.002
Comorbidities						
Cancer (+/–)	323 (16.2)	45,839 (9.1)	2.04 (1.80–1.30)	<0.001	1.35 (1.20–1.53)	<0.001
COPD (+/–)	569 (28.5)	41,694 (8.3)	4.56 (4.13–5.04)	<0.001	2.85 (2.56–3.18)	<0.001
CHF (+/–)	471 (23.6)	44,346 (8.8)	3.25 (2.93–3.62)	<0.001	1.26 (1.12–1.43)	<0.001
Dementia (+/–)	253 (12.7)	22,741 (4.5)	3.14 (2.75–3.60)	<0.001	1.61 (1.40–1.86)	<0.001
Liver disease (+/–)	242 (12.1)	30,387 (6.0)	2.23 (1.94–2.56)	<0.001	1.37 (0.95–1.97)	0.094
Renal disease (+/–)	648 (32.5)	54,398 (10.8)	4.06 (3.68–4.47)	<0.001	2.12 (1.89–2.37)	<0.001
Model II						
ACCI score, mean ±SD	5.2 ±2.7	4.0 ±2.9	1.25 (1.24–1.27)	<0.001	1.25 (1.24–1.27)	<0.001
Model III						
ACCI score						
0–3	527 (24.4)	255,035 (50.5)	Reference		Reference	
4–6	859 (43.1)	157,045 (31.1)	4.52 (3.91–5.22)	<0.001	4.44 (3.84–5.12)	<0.001
7–9	485 (24.3)	65,335 (12.9)	9.73 (8.41–11.25)	<0.001	9.46 (8.17–10.95)	<0.001
10+	123 (6.2)	27,775 (5.5)	10.45 (8.82–12.37)	<0.001	10.00 (8.43–11.85)	<0.001

ACCI: Age-adjusted Charlson comorbidity index, AOR: Adjusted odds ratio, CHF: Congestive heart failure, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, OR: Odds ratio, SD: Standard deviation, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus
Model I: Included age, sex, diabetes, and comorbidities. Adjusted estimates of age, sex, diabetes, and comorbidities are reported only for this model.
Model II: Included ACCI (continuous variable) sex, and diabetes. Adjusted estimates are reported only for ACCI.
Model III: Included ACCI (categorical variable), sex, and diabetes. Adjusted estimates are reported only for ACCI.

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The overall 30-day mortality following IRI hospitalization was 8%, while 90-day mortality was 10%. Both 30- and 90-day mortality rates were significantly higher in older ($p = 0.003$, $p < 0.001$) people and those with a higher cumulative comorbidity score (<0.001 each). In addition, 30- and 90-day mortality rates were significantly higher in people with CHF ($p = 0.013$, $p < 0.001$), dementia ($p = 0.026$, $p = 0.008$), and renal disease ($p = 0.059$, $p = 0.004$) compared with those without these comorbidities (172). See table 4.1.2 for details.

In survival regression, advanced age significantly elevated the risk of 90-day mortality, as did the presence of CHF and renal disease. Each score point increase in the cumulative comorbidity score elevated the mortality risk by 14%. However, COPD, types of diabetes, and types of influenza had no significant impact on mortality (172). See table 4.1.3 for details.

Table 4.1.2. Cumulative mortality after hospitalization for influenza-related illness (N = 1994) (172).

Variables	30-days mortality		90-days mortality	
	% (95%CI)	P-value	% (95%CI)	P-value
Overall	7.9 (6.8–9.1)	NA	10.3 (9.0–11.7)	NA
Age categories				
<50 years	1.7 (0.4–6.4)		1.7 (0.4–6.4)	
50–59 years	5.2 (2.8–9.8)		5.2 (2.8–9.8)	
60–69 years	6.7 (4.6–9.7)	0.003	8.3 (5.9–11.6)	<0.001
70–79 years	7.5 (5.6–9.8)		9.7 (7.6–12.2)	
80+ years	10.7 (8.5–13.3)		14.9 (12.4–17.8)	
Sex				
Male	8.7 (7.1–10.5)	0.219	10.9 (9.2–12.9)	0.385
Female	6.9 (5.5–8.8)		9.6 (7.8–11.7)	
Type of diabetes				
T1DM	3.7 (1.5–8.6)	0.059	5.9 (3.0–11.4)	0.081
T2DM	8.2 (7.0–9.5)		10.6 (9.3–12.1)	
Type of influenza				
J09	8.1 (5.8–11.1)		9.2 (6.8–12.4)	
J10	8.8 (7.2–10.7)	0.166	11.5 (9.7–13.7)	0.169
J11	6.2 (4.5–8.5)		8.8 (6.8–11.5)	
Comorbidities				
Cancer				
No	7.7 (6.5–9.1)	0.560	9.7 (8.4–11.2)	0.054
Yes	8.7 (6.1–12.3)		13.3 (10.1–17.5)	
COPD				
No	7.9 (6.6–9.4)	0.866	9.9 (8.5–11.6)	0.467
Yes	7.9 (6.0–10.5)		11.1 (8.8–13.9)	
CHF				
No	7.1 (5.9–8.5)	0.013	8.9 (7.6–10.5)	<0.001
Yes	10.4 (8.0–13.5)		14.7 (11.8–18.2)	
Dementia				
No	7.3 (6.2–8.7)	0.026	9.6 (8.3–11.1)	0.008
Yes	11.5 (8.1–16.1)		15.0 (11.2–20.1)	
Liver disease				
No	7.9 (6.7–9.2)	0.967	10.5 (9.1–12.0)	0.528
Yes	7.8 (5.1–12.0)		9.1 (6.1–13.5)	
Renal disease				
No	7.1 (5.8– 8.6)	0.059	8.9 (7.5–10.6)	0.004
Yes	9.6 (7.5–12.1)		13.1 (10.7–16.0)	
ACCI score				
0–3	3.4 (2.2–5.4)		3.6 (2.3–5.6)	
4–6	9.4 (7.7–11.6)		11.5 (9.6–13.9)	
7–9	9.5 (7.2–12.5)	<0.001	13.6 (10.9–17.0)	<0.001
10+	9.8 (5.7–16.5)		17.1 (11.5–25.0)	

ACCI: Age-adjusted Charlson comorbidity index, CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, CHF: Congestive heart failure, NA: Not available, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus. P-values are reported for log-rank test.

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Table 4.1.3. Simple and multiple multilevel survival regression for 90-days all-cause mortality related to influenza-related illness (N = 1994) (172).

Variables	Simple regression		Multiple regression	
	HR (95%CI)	P-value	AHR (95%CI)	P-value
Model I				
Age categories				
<50 years	Reference		Reference	
50–59 years	3.25 (0.70–15.04)	0.132	3.00 (0.65–13.94)	0.161
60–69 years	5.18 (1.24–21.64)	0.024	4.16 (0.99–17.55)	0.052
70–79 years	6.06 (1.48–24.75)	0.012	4.79 (1.16–19.76)	0.030
80+ years	9.41 (2.32–38.23)	0.002	7.15 (1.74–29.46)	0.006
Sex				
Male	Reference		Reference	
Female	0.85 (0.64–1.13)	0.262	0.79 (0.60–1.05)	0.103
Type of diabetes				
T1DM	Reference		Reference	
T2DM	1.80 (0.89–3.67)	0.682	1.40 (0.68–2.88)	0.358
Type of influenza				
J09	Reference		Reference	
J10	1.23 (0.85–1.77)	0.273	1.24 (0.86–1.78)	0.248
J11	0.92 (0.59–1.42)	0.696	0.97 (0.64–1.48)	0.890
Comorbidities				
Cancer (+/–)	1.46 (1.03–2.05)	0.031	1.41 (1.00–1.98)	0.051
COPD (+/–)	1.11 (0.82–1.50)	0.484	1.06 (0.78–1.44)	0.702
CHF (+/–)	1.68 (1.25–2.25)	0.001	1.97 (1.31–2.98)	0.001
Dementia (+/–)	1.61 (1.13–2.29)	0.009	1.24 (0.86–1.80)	0.250
Liver disease (+/–)	0.88 (0.57–1.38)	0.584	0.85 (0.54–1.33)	0.468
Renal disease (+/–)	1.49 (1.13–1.98)	0.005	1.50 (1.05–2.14)	0.026
Model II				
ACCI score	1.14 (1.08–1.19)	<0.001	1.14 (1.08–1.19)	<0.001

ACCI: Age-adjusted Charlson comorbidity index, AHR: Adjusted hazard ratio, COPD: Chronic obstructive pulmonary disease, CHF: Congestive heart failure, HR: Hazard ratio, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus
Model I: Included age, sex, diabetes, and comorbidities.
Model II: Included ACCI (continuous variable), sex, and diabetes. Adjusted estimates are reported only for ACCI.

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4.2. Adverse outcomes of COVID-19 in hospitalized patients with diabetes mellitus

In this cohort of 40,602 patients hospitalized for COVID-19, the majority were 60 to 89 years old, and 52% were men. The most common comorbidity was CVD (33%), followed by hypertension (26%), renal disease (8%), COPD (6%), and dementia (5%), respectively. Of the hospitalized patients, 12% had diabetes, 15% were admitted to ICU, and 16% died in the hospital (173).

In the entire cohort, patients with diabetes were significantly older ($p < 0.001$) and more males (57% vs. 52%, $p < 0.001$). Comorbidities such as CVD (78% vs. 27%, $p < 0.001$), COPD (11% vs. 5%, $p < 0.001$), dementia (10% vs. 4%, $p < 0.001$), liver disease (7% vs. 2%, $p < 0.001$), chronic renal disease (22% vs. 6%, $p < 0.001$), cancer (5% vs. 3%, $p < 0.001$), and hypothyroidism (7% vs. 3%, $p < 0.001$) were more common in patients with diabetes compared with those without diabetes. Regarding adverse outcomes, both ICU admission (18% vs. 14%, $p < 0.001$) and in-hospital mortality (19% vs. 16%, $p < 0.001$) were significantly higher in patients with diabetes than those without diabetes. In the PSM cohort generated from the overall cohort, patients with diabetes (N = 4971) were well-matched with patients without diabetes (N = 4971) in terms of characteristics and comorbidities (173).

The impact of diabetes on adverse outcomes in patients with COVID-19 was assessed in both unmatched and PSM cohorts. In the unmatched cohort, patients with diabetes were 36% ($p < 0.001$) more likely to be admitted to ICU compared to patients without diabetes. In the PSM cohort, this association was attenuated to 15% ($p = 0.009$); notwithstanding remained significant (173). See table 4.2.1 for details. In the unmatched cohort, patients with diabetes had 24% ($p < 0.001$) higher odds of in-hospital mortality compared to those without diabetes. However, this association was attenuated to 8% ($p = 0.146$) in the PSM cohort and became insignificant (173). See table 4.2.2 for details.

Table 4.2.1. Comparison and simple logistic regression analysis of ICU admission with diabetes and other variables in unmatched and propensity score matched cohorts (173).

Variables	Unmatched cohort				PSM cohort	
	No ICU Admission (N = 34,634)	ICU Admission (N = 5968)	Logistic regression		Logistic regression	
	n (%)	n (%)	OR (95%CI)	P-value	OR (95%CI)	P-value
Diabetes	4060 (11.7)	911 (15.3)	1.36 (1.25–1.47)	<0.001	1.15 (1.04–1.28)	0.009
Men	17,430 (50.3)	3848 (64.5)	Reference	<0.001	Reference	<0.001
Women	17,204 (49.7)	2120 (35.5)	0.56 (0.53–0.59)		0.56 (0.50–0.63)	
Age categories						
20–29 years	982 (2.8)	75 (1.3)	Reference		Reference	
30–39 years	1427 (4.1)	149 (2.5)	1.37 (1.03–1.83)	0.033	0.33 (0.09–1.47)	0.138
40–49 years	2161 (6.2)	318 (5.3)	1.92 (1.49–2.52)	<0.001	0.60 (0.18–2.39)	0.441
50–59 years	4456 (12.9)	907 (15.2)	2.66 (2.10–3.43)	<0.001	0.54 (0.17–2.11)	0.348
60–69 years	5261 (15.2)	1413 (23.7)	3.51 (2.77–4.51)	<0.001	0.65 (0.20–2.54)	0.507
70–79 years	7838 (22.6)	1826 (30.6)	3.04 (2.41–3.90)	<0.001	0.48 (0.15–1.89)	0.271
80–89 years	9365 (27.0)	1167 (19.6)	1.63 (1.29–2.09)	<0.001	0.22 (0.07–0.86)	0.032
90+ years	3144 (9.1)	113 (1.9)	0.47 (0.35–0.64)	<0.001	0.04 (0.01–0.17)	<0.001
Comorbidities						
Myocardial infarction	239 (0.7)	101 (1.7)	2.48 (1.95–3.12)	<0.001	2.27 (1.68–3.04)	<0.001
Cardiac arrhythmias	3612 (10.4)	623 (10.4)	1.00 (0.91–1.09)	0.978	0.93 (0.82–1.05)	0.267
Valvular heart diseases	844 (2.4)	146 (2.5)	1.00 (0.84–1.20)	0.957	0.87 (0.69–1.10)	0.255
Hypertension	8929 (25.8)	1609 (27.0)	1.06 (1.00–1.13)	0.056	1.09 (0.98–1.22)	0.112
Congestive heart failure	1676 (4.8)	335 (5.6)	1.17 (1.04–1.32)	0.012	1.25 (1.08–1.45)	0.004
Peripheral vascular disease	1119 (3.2)	222 (3.7)	1.16 (1.00–1.34)	0.054	1.07 (0.89–1.28)	0.449
Stroke	1024 (3.0)	188 (3.1)	1.07 (0.91–1.25)	0.416	1.01 (0.83–1.22)	0.915
COPD	1854 (5.3)	417 (7.0)	1.33 (1.19–1.48)	<0.001	1.23 (1.05–1.43)	0.010
PCD	436 (1.3)	155 (2.6)	2.09 (1.73–2.51)	<0.001	2.19 (1.65–2.87)	<0.001
Dementia	1975 (5.7)	75 (1.3)	0.21 (0.17–0.26)	<0.001	0.14 (0.09–0.20)	<0.001
Rheumatoid disorders	227 (0.7)	27 (0.5)	0.69 (0.45–1.01)	0.059	0.65 (0.37–1.07)	0.097
Peptic ulcer disease	68 (0.2)	18 (0.3)	1.55 (0.89–2.55)	0.116	1.08 (0.46–2.21)	0.852
Liver disease	848 (2.5)	183 (3.1)	1.26 (1.07–1.48)	0.006	1.15 (0.94–1.40)	0.173
Paralysis	105 (0.3)	21 (0.3)	1.17 (0.71–1.83)	0.524	1.68 (0.92–2.91)	0.091
Other neurological disorders	970 (2.8)	101 (1.7)	0.60 (0.48–0.73)	<0.001	0.56 (0.40–0.75)	<0.001
Renal disease	2741 (7.9)	432 (7.2)	0.91 (0.82–1.01)	0.071	0.81 (0.71–0.93)	0.002
Cancer	1128 (3.3)	190 (3.2)	0.98 (0.83–1.14)	0.774	1.00 (0.78–1.27)	0.999
Hypothyroidism	1157 (3.3)	178 (3.0)	0.89 (0.76–1.04)	0.150	0.93 (0.75–1.14)	0.508
Coagulation disorders	167 (0.5)	85 (1.4)	2.98 (2.29–3.87)	<0.001	4.06 (2.81–5.85)	<0.001
Fluid & electrolyte disorders	1280 (3.7)	153 (2.6)	0.69 (0.58–0.81)	<0.001	0.89 (0.71–1.11)	0.292
Deficiency anemia	328 (0.9)	43 (0.7)	0.76 (0.55–1.04)	0.084	0.72 (0.46–1.09)	0.123
Alcohol abuse	188 (0.5)	53 (0.9)	1.65 (1.20–2.22)	0.002	1.54 (1.01–2.29)	0.046
Psychosis	126 (0.4)	25 (0.4)	1.16 (0.74–1.75)	0.511	1.12 (0.58–1.99)	0.721
Depression	937 (2.7)	137 (2.3)	0.85 (0.70–1.01)	0.065	0.93 (0.72–1.18)	0.552
Charlson comorbidity index						
0	25,868 (74.7)	4493 (75.3)	Reference		Reference	
1–2	6383 (18.4)	1101 (18.4)	0.99 (0.92–1.07)	0.851	0.90 (0.80–1.01)	0.083
3–4	1885 (5.4)	301 (5.0)	0.92 (0.81–1.04)	0.188	0.84 (0.72–0.99)	0.035
5+	498 (1.4)	73 (1.2)	0.85 (0.65–1.08)	0.176	0.79 (0.58–1.05)	0.110

CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, ICU: Intensive care unit, OR: Odds ratio, PCD: Pulmonary circulation disorders, PSM: Propensity score matched

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Table 4.2.2. Comparison and simple logistic regression analysis of in-hospital mortality with diabetes and other variables in unmatched and propensity score-matched cohorts (173).

Variables	Unmatched cohort				PSM	
	Survived (N = 34,033)	Died (N = 6569)	Logistic regression		Logistic regression	
	n (%)	n (%)	OR (95%CI)	P-value	OR (95%CI)	P-value
Diabetes	4034 (11.9)	937 (14.3)	1.24 (1.15–1.34)	<0.001	1.08 (0.97–1.19)	0.146
Men	17,582 (51.7)	3696 (56.3)	Reference		Reference	
Women	16,451 (48.3)	2873 (43.7)	0.83 (0.79–0.88)	<0.001	0.84 (0.76–0.94)	<0.001
Age categories						
20–29 years	1048 (3.1)	9 (0.1)	Reference		Reference	
30–39 years	1557 (4.6)	19 (0.3)	1.41 (0.65–3.31)	0.396	0.14 (0.00–5.63)	0.250
40–49 years	2444 (7.2)	35 (0.5)	1.65 (0.82–3.68)	0.168	0.12 (0.01–3.42)	0.165
50–59 years	5173 (15.2)	190 (2.9)	4.20 (2.28–8.92)	<0.001	0.46 (0.09–11.60)	0.533
60–69 years	6093 (17.9)	581 (8.8)	10.90 (5.99–22.90)	<0.001	1.05 (0.20–25.80)	0.965
70–79 years	8018 (23.6)	1646 (25.1)	23.50 (12.9–49.20)	<0.001	2.23 (0.43–54.80)	0.402
80–89 years	7727 (22.7)	2805 (42.7)	41.50 (22.9–87.00)	<0.001	3.75 (0.72–92.40)	0.133
90+ years	1973 (5.8)	1284 (19.5)	74.40 (40.9–157.00)	<0.001	6.80 (1.30–168.00)	0.019
ICU admission	3991 (11.7)	1977 (30.1)	3.24 (3.04–3.45)	<0.001	3.09 (2.75–3.47)	<0.001
Comorbidities						
Myocardial infarction	219 (0.6)	121 (1.8)	2.90 (2.31–3.62)	<0.001	2.27 (1.69–3.03)	<0.001
Cardiac arrhythmias	3160 (9.3)	1075 (16.4)	1.91 (1.77–2.06)	<0.001	1.78 (1.60–1.99)	<0.001
Valvular heart disease	751 (2.2)	239 (3.6)	1.67 (1.44–1.94)	<0.001	1.62 (1.32–1.96)	<0.001
Hypertension	8737 (25.7)	1801 (27.4)	1.09 (1.03–1.16)	0.003	0.75 (0.68–0.84)	<0.001
Congestive heart failure	1326 (3.9)	685 (10.4)	2.87 (2.61–3.16)	<0.001	2.72 (2.38–3.10)	0.000
Peripheral vascular disease	962 (2.8)	379 (5.8)	2.11 (1.86–2.38)	<0.001	1.78 (1.52–2.09)	<0.001
Stroke	887 (2.6)	325 (4.9)	1.95 (1.71–2.21)	<0.001	1.75 (1.47–2.07)	<0.001
COPD	1815 (5.3)	456 (6.9)	1.32 (1.19–1.47)	<0.001	1.20 (1.03–1.40)	0.022
PCD	457 (1.3)	134 (2.0)	1.53 (1.26–1.85)	<0.001	1.67 (1.24–2.21)	0.001
Dementia	1344 (3.9)	706 (10.7)	2.93 (2.66–3.22)	<0.001	2.32 (2.00–2.68)	<0.001
Rheumatoid disease	205 (0.6)	49 (0.7)	1.24 (0.90–1.68)	0.183	1.37 (0.90–2.03)	0.140
Peptic ulcer disease	66 (0.2)	20 (0.3)	1.58 (0.93–2.56)	0.087	1.33 (0.62–2.61)	0.443
Liver disease	847 (2.5)	184 (2.8)	1.13 (0.96–1.32)	0.144	1.03 (0.84–1.26)	0.782
Paralysis	100 (0.3)	26 (0.4)	1.35 (0.86–2.06)	0.183	0.77 (0.35–1.49)	0.456
Other neurological disorders	774 (2.3)	297 (4.5)	2.04 (1.77–2.33)	<0.001	2.13 (1.72–2.62)	<0.001
Renal disease	2243 (6.6)	930 (14.2)	2.34 (2.15–2.54)	<0.001	2.28 (2.04–2.55)	<0.001
Cancer	965 (2.8)	353 (5.4)	1.95 (1.72–2.20)	<0.001	1.86 (1.51–2.28)	<0.001
Hypothyroidism	1171 (3.4)	164 (2.5)	0.72 (0.61–0.85)	<0.001	0.60 (0.47–0.76)	<0.001
Coagulation disorders	193 (0.6)	59 (0.9)	1.59 (1.18–2.12)	0.003	1.66 (1.09–2.47)	0.020
Fluid & electrolyte disorders	1172 (3.4)	261 (4.0)	1.16 (1.01–1.33)	0.036	1.19 (0.97–1.45)	0.094
Deficiency anaemia	307 (0.9)	64 (1.0)	1.08 (0.82–1.41)	0.567	0.84 (0.55–1.24)	0.394
Alcohol abuse	177 (0.5)	64 (1.0)	1.88 (1.40–2.50)	<0.001	1.44 (0.94–2.14)	0.090
Psychosis	125 (0.4)	26 (0.4)	1.08 (0.69–1.63)	0.714	1.15 (0.61–2.01)	0.651
Depression	907 (2.7)	167 (2.5)	0.95 (0.80–1.12)	0.575	0.84 (0.65–1.07)	0.161
Charlson Comorbidity Index						
0	26,400 (77.6)	3961 (60.3)	Reference		Reference	
1–2	5752 (16.9)	1732 (26.4)	2.01 (1.88–2.14)	<0.001	2.47 (2.18–2.80)	<0.001
3–4	1491 (4.4)	695 (10.6)	3.11 (2.82–3.42)	<0.001	3.99 (3.44–4.62)	<0.001
5+	390 (1.1)	181 (2.76)	3.09 (2.58–3.70)	<0.001	4.40 (3.45–5.59)	<0.001

CI: Confidence interval, COPD: Chronic obstructive pulmonary disease, ICU: Intensive care unit, OR: Odds ratio, PCD: Pulmonary circulation disorders, PSM: Propensity score matched

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4.3. Biomarkers of in-hospital mortality in COVID-19 patients with diabetes mellitus

A total of 747 people with T2DM (71%), prediabetes (15%), other types of diabetes (9%), and T1DM (6%) were included in this analysis. Approximately 19% of patients died in the hospital, and they were significantly older and had a higher burden of comorbidities such as hypertension, CVD, chronic kidney disease, and cancer as compared to those who were discharged alive (174).

In inflammatory biomarkers, CRP ($p < 0.001$), IL-6 ($p < 0.001$), and PCT ($p < 0.001$) were significantly elevated in patients who died compared with those discharged alive. Whereas LDH ($p = 0.147$) and ferritin ($p = 0.559$) levels were similar between those who died and were alive. Hepatic markers such as AST ($p = 0.027$), ALT ($p = 0.037$), and AST–ALT ratio ($p < 0.001$) were found elevated in non-survivors versus survivors. Likewise, the coagulation marker D-dimer ($p < 0.001$) was elevated in non-survivors compared with survivors. Both cardiac biomarkers NT-proBNP ($p < 0.001$) and Troponin T ($p < 0.001$) were also elevated in those who died compared with those who survived (174). See figure 4.3.1 for details.

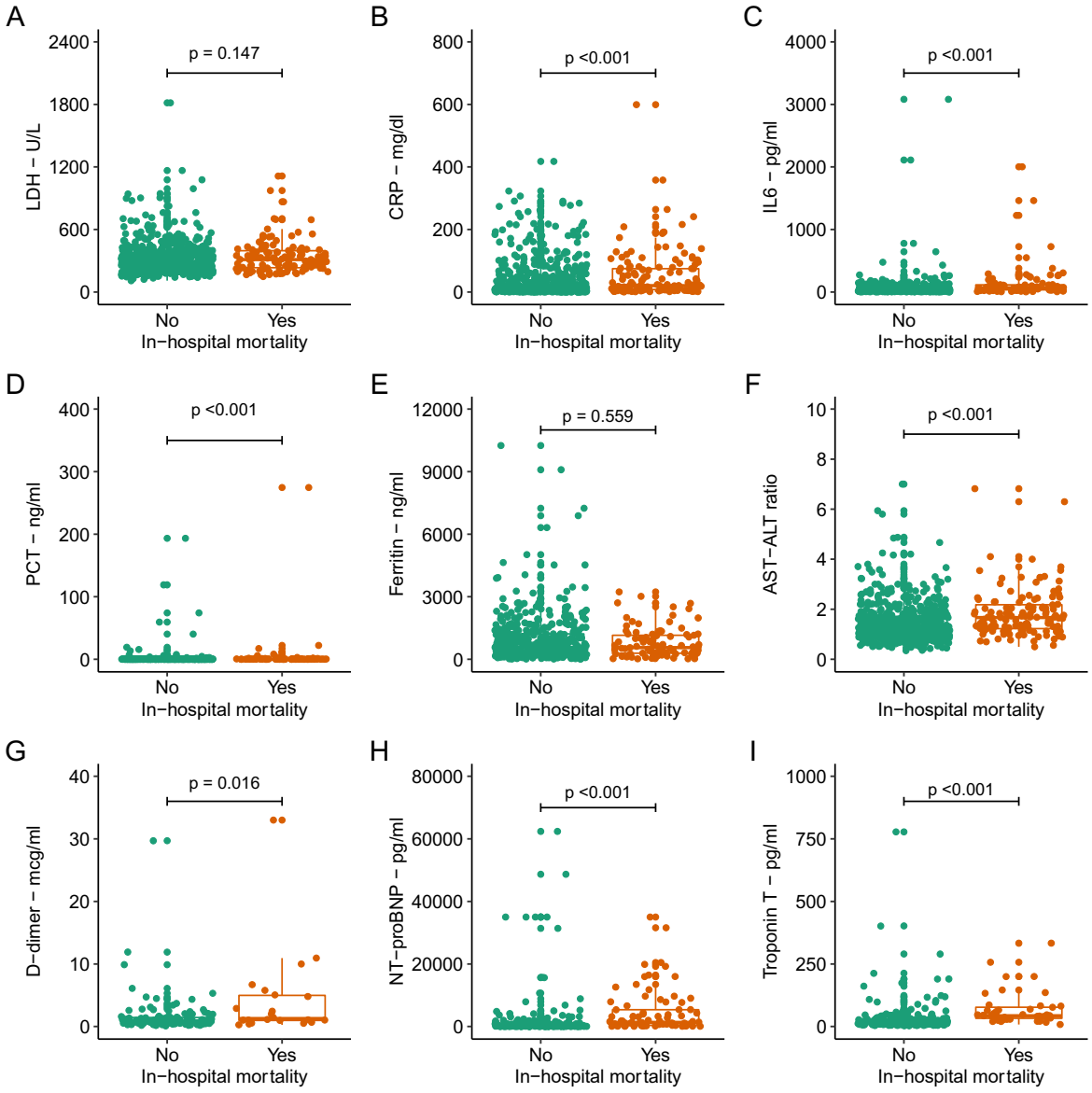

Figure 4.3.1. Distribution of biomarkers by in-hospital mortality (174).

AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, CRP: C-Reactive Protein, IL6: Interleukin 6, LDH: Lactate Dehydrogenase, NT-proBNP: N terminal pro brain natriuretic peptide, PCT: Procalcitonin, P: Wilcoxon rank-sum test p-value

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Of all biomarkers, CRP, IL-6, PCT, AST–ALT ratio, NT-proBNP, and Troponin T were found to be significantly associated with in-hospital mortality after accounting for age, sex, and type of diabetes. The association of both cardiac biomarkers remained significant with mortality after excluding patients with existing CHF (174). See table 4.3.1 for details.

Table 4.3.1. Logistic regression analysis of biomarkers with in-hospital mortality (174).

Biomarkers	Simple logistic regression			Multiple logistic regression		
	OR	95%CI	P-value	AOR	95%CI	P-value
Inflammatory biomarkers						
LDH – U/L	1.40	0.88 – 2.25	0.158	2.03	1.21 – 3.42	0.008
CRP – mg/dl	1.30	1.15 – 1.47	<0.001	1.33	1.16 – 1.52	<0.001
IL-6 – pg/ml	1.66	1.34 – 2.06	<0.001	1.60	1.27 – 2.01	<0.001
PCT – ng/ml	1.31	1.13 – 1.51	<0.001	1.25	1.06 – 1.48	0.007
Ferritin – ng/ml	0.90	0.74 – 1.10	0.300	1.07	0.86 – 1.35	0.541
Coagulation biomarkers						
D-dimer – mcg/ml	1.93	1.22 – 3.03	0.005	1.66	0.97 – 2.82	0.063
Hepatic biomarkers						
AST-ALT ratio	3.00	1.97 – 4.56	<0.001	1.89	1.19 – 3.01	0.007
Cardiac biomarkers						
NT-proBNP – pg/ml	1.59	1.35 – 1.86	<0.001	1.50	1.24 – 1.80	<0.001
Troponin T – pg/ml	2.78	1.90 – 4.07	<0.001	2.20	1.44 – 3.35	<0.001

AOR: Adjusted Odds Ratio, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, CI: Confidence Interval, CRP: C-Reactive Protein, IL6: Interleukin 6, NT-proBNP: N terminal pro brain natriuretic peptide, OR: Odds Ratio, PCT: Procalcitonin
Multiple logistic regression model was adjusted for age, sex, and types of diabetes.

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Of all studied biomarkers, only cardiac biomarkers such as NT-proBNP (AUC = 0.74) and Troponin T (AUC = 0.81) exhibited good and excellent discrimination, respectively (174). See figure 4.3.2 for details.

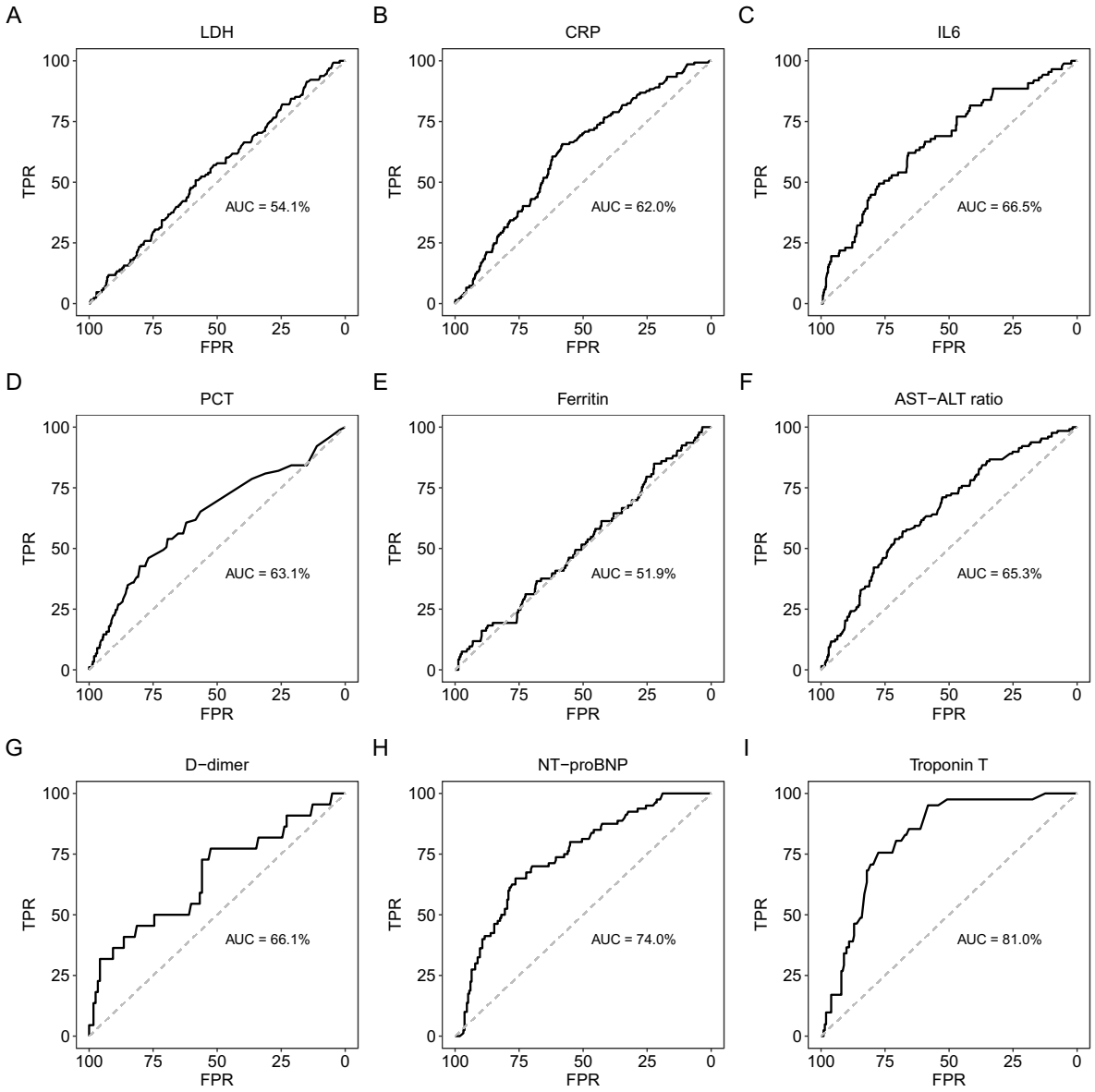

Figure 4.3.2. ROC curves of biomarkers (174).

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, AUC: Area Under the Curve, CRP: C-Reactive Protein, FPR: False Positive Rate, IL6: Interleukin 6, LDH: Lactate Dehydrogenase, NT-proBNP: N terminal pro brain natriuretic peptide, PCT: Procalcitonin, TPR: True Positive Rate

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Calibration was satisfactory for NT-proBNP (H-L statistics = 9.50, p = 0.302). While it unsatisfactory for Troponin T (H-L statistics = 20.03, p = 0.010) (174). See table 4.3.2 for details.

Table 4.3.2. Hosmer-Lemeshow goodness of fit test of biomarkers (174).

Biomarkers	**Hosmer-Lemeshow Test**	
	Statistics	P-value
Inflammatory biomarkers		
LDH – U/L	3.22	0.920
CRP – mg/dl	5.82	0.667
IL6 – pg/ml	3.81	0.874
PCT – ng/ml	6.63	0.577
Ferritin – ng/ml	6.42	0.600
Coagulation biomarkers		
D-dimer – mcg/ml	6.19	0.626
Hepatic biomarkers		
AST-ALT ratio	7.96	0.437
Cardiac biomarkers		
NT-proBNP – pg/ml	9.50	0.302
Troponin T – pg/ml	20.03	0.010

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, CRP: C-Reactive Protein, IL6: Interleukin 6, LDH: Lactate Dehydrogenase, NT-proBNP: N terminal pro brain natriuretic peptide, PCT: Procalcitonin

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4.4. Performance of SAPS 3 in critically ill COVID-19 patients with and without diabetes mellitus

Of 5850 patients admitted to ICU, 29% had diabetes, 67% were males, and 75% were aged ≥60 years. The overall mean SAPS 3 score was 57 ±13 and was significantly higher in patients with diabetes than in those without it (59 ±13 vs. 57 ±13, p <0.001) (175).

The overall in-hospital mortality was 39% and was significantly higher in patients with diabetes (43% vs. 37%, p <0.001) than in those without diabetes. Patients who died had a higher SAPS 3 score than those who were discharged alive (63 ±13 vs. 54 ±12, p <0.001). Similar results were noted in diabetes (64 ±13 vs. 55 ±11, p <0.001) and non-diabetes patients (62 ±13 vs. 54 ±13, p <0.001), respectively (175).

The in-hospital mortality predicted by the standard, Central European, and Austrian equations of SAPS 3 were 33% ±22%, 28% ±21%, and 38% ±21%, respectively. All three versions of SAPS 3 predicted significantly higher mortality (p <0.001 each) in diabetes patients compared to those without diabetes. However, both standard and Central European equations underpredicted the mortality in the entire, diabetes, and non-diabetes cohorts. The SMR estimated by the Austrian equation showed good concordance with the observed mortality in the overall and non-diabetes cohorts, but underpredicted the mortality in the diabetes cohort (175). See table 4.4.1 for details.

The discriminatory performance of SAPS 3 was unsatisfactory for all three equations (AUC = 0.69 each). The AUC of SAPS 3 was insignificantly higher (p = 0.193) in patients with diabetes (AUC = 0.70) compared to non-diabetes patients (AUC = 0.68) for each equation. The Brier score was >0.20 for all three equations in all cohorts, indicating the poor accuracy of SAPS 3 in COVID-19 patients (175). See table 4.4.1 for details.

Table 4.4.1. Performance of SAPS 3 standard, Central Europe, and Austrian equations in predicting in-hospital mortality in all, diabetes, and non-diabetes patients (175).

SAPS 3 Equations	Mortality		Discrimination	Calibration	
	Predicted Mortality (Mean ±SD)	SMR (95%CI)	AUROC (95%CI)	H-L X^2, p-Value	Brier score
Standard equation					
All	32.47 ±21.69	1.20 (1.16–1.24)	68.67 (67.31–70.02)	100.03, <0.001	0.22
Diabetes	34.56 ±21.62	1.24 (1.18–1.31)	70.03 (67.53–72.53)	12.21, 0.142	0.22
No diabetes	31.63 ±21.66	1.18 (1.13–1.22)	68.05 (66.44–69.67)	101.64, <0.001	0.22
Central Europe equation					
All	28.05 ±21.43	1.39 (1.34–1.43)	68.67 (67.31–70.02)	120.95, <0.001	0.23
Diabetes	30.02 ±21.56	1.43 (1.35–1.51)	70.03 (67.53–72.53)	15.08, 0.058	0.23
No diabetes	27.28 ±21.33	1.37 (1.31–1.42)	68.05 (66.44–69.67)	119.99, <0.001	0.23
Austrian equation					
All	37.86 ±20.56	1.03 (0.99–1.06)	68.67 (67.31–70.02)	65.10, <0.001	0.22
Diabetes	40.03 ±20.16	1.07 (1.02–1.13)	70.03 (67.53–72.53)	9.04, 0.339	0.22
No diabetes	37.00 ±20.66	1.01 (0.98–1.05)	68.05 (66.44–69.67)	69.55, <0.001	0.22

AUROC: Area under the receiver operating characteristic curve, CI: Confidence interval, H-L X^2: Hosmer–Lemeshow Chi-square test, SAPS 3: Simplified Acute Physiology Score 3, SMR: Standardized Mortality Ratio

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Both standard and Central European equations of SAPS 3 were miscalibrated in all three cohorts. Specifically, these equations underpredicted the mortality in low- and medium-risk groups in all cohorts, while overpredicted the mortality in high-risk groups in all and non-diabetes patients. In contrast, the Austrian equation showed good calibration in low- and medium-risk groups in all and non-diabetes patients, but overpredicted the mortality in high-risk groups. While this equation showed satisfactory calibration across all risk groups of diabetes patients (175). See figure 4.4.1 for details.

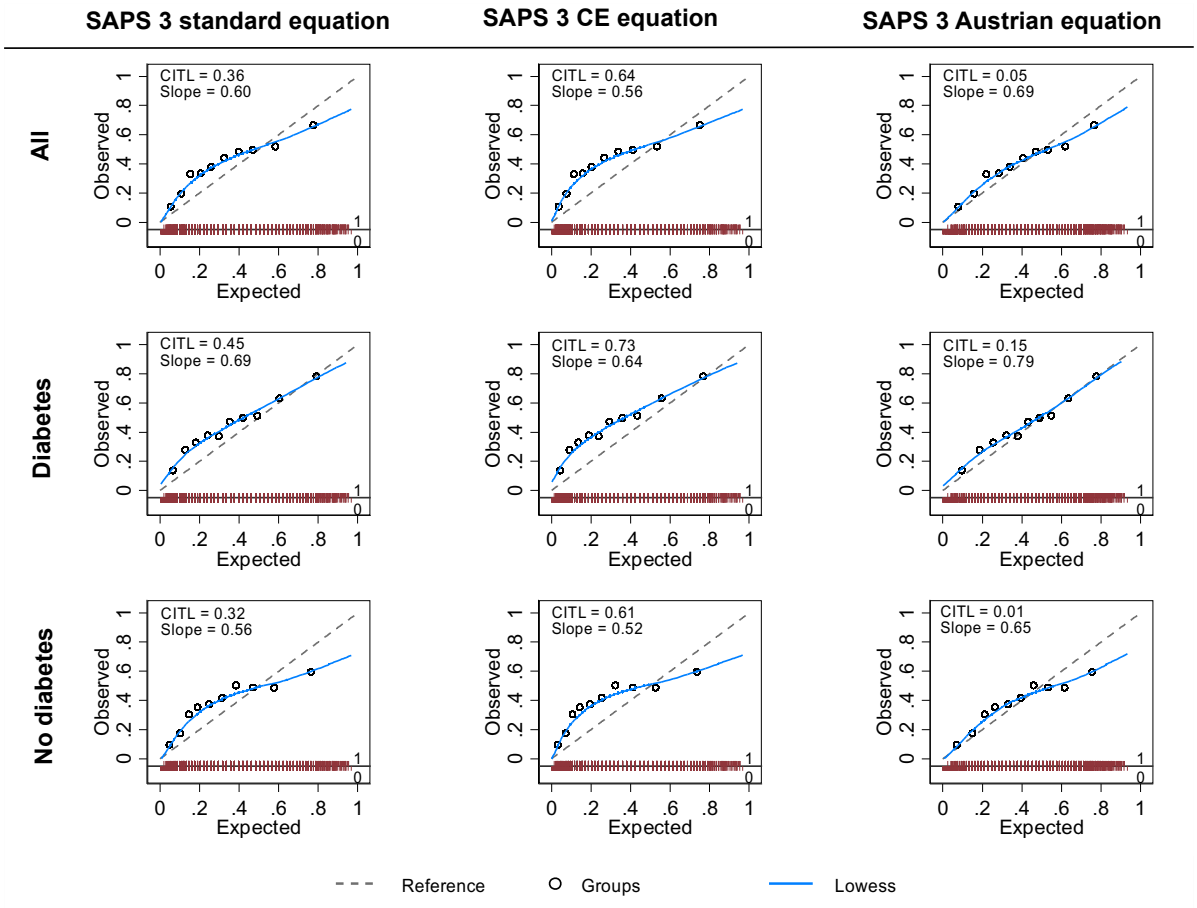

Figure 4.4.1. Calibration plots for SAPS 3 standard, SAPS 3 Central Europe, and SAPS 3 Austrian equations in all, diabetes, and non-diabetes patients with COVID-19 (175).

CE: Central Europe, CITL: Calibration in-the-large, SAPS 3: Simplified Acute Physiology Score 3, Slope: Calibration slope

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4.5. Humoral immune response to COVID-19 vaccination in people with diabetes mellitus

A total of 150 participants with T1DM (N = 75) and T2DM (N = 75) were included in the analysis. Three participants were hospitalized after vaccination due to peripheral oedema, chronic heart failure, grade 3 atrioventricular block, or miscarriage. No cases of severe anaphylaxis were reported during the study. Common side effects reported after the first and second vaccination were injection site reactions (87% and 63%), pain at the injection site (66% and 61%), fatigue (38% and 34%), and headache (28% and 28%). These side effects were experienced less frequently by participants with T2DM than those with T1DM, particularly after the second vaccination (176).

After the first vaccination, the median (IQR) level of Anti-SARS-CoV-2 S RBD antibodies was 1.1 (8.1) with 53% above the detection limit of 0.8 in participants with T1DM and 0.3 (2.4) with 48% above the detection limit in participants with T2DM. After the second vaccination, the participants with T1DM had the highest antibody levels compared with T2DM ($p = 0.013$) and healthy people ($p = 0.022$). However, these differences became insignificant after accounting for age and sex and correcting for multiple comparisons (176). See figure 4.5.1A for details.

Investigation of the impact of glycaemic control on antibody response after COVID-19 vaccination showed that antibody levels were not significantly different ($p = 0.249$ and $p = 1.000$) between well controlled (HbA1c ≤58 mmol/mol [≤7.5%]) and insufficiently controlled (HbA1c >58 mmol/mol [>7.5%]) groups within T1DM and T2DM participants each. Likewise, comparisons between types of diabetes showed no significant differences in antibody levels after adjusting for age, sex, BMI, and eGFR and correcting for multiple comparisons (176). See figure 4.5.1 A & B for details.

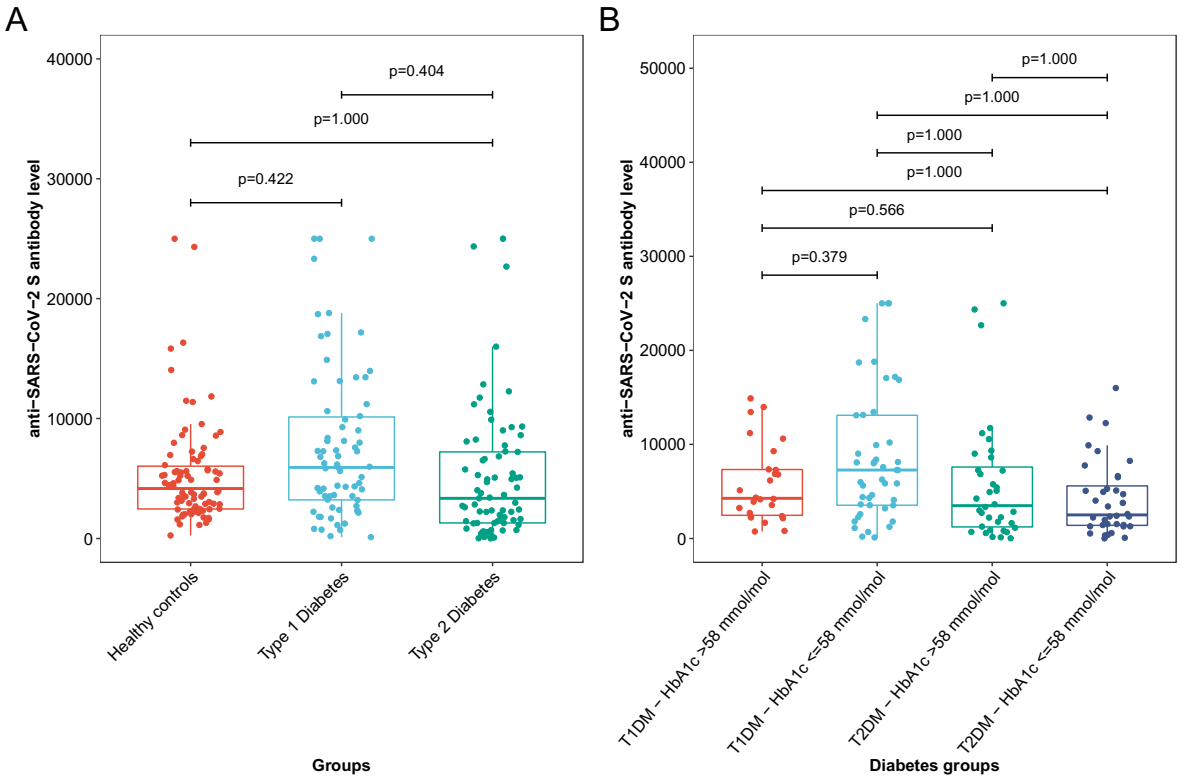

Figure 4.5.1. A: Comparison of anti-SARS-CoV-2-S antibodies between participants with diabetes and healthy controls after the second vaccination. B: Comparison of anti-SARS-CoV-2 S antibodies in people with type 1 and type 2 diabetes and HbA1c level of ≤58 mmol/mol (176).

HbA1c: Glycated haemoglobin, T1DM: Type 1 diabetes mellitus, T2DM; Type 2 diabetes mellitus, P: P-value. P values are adjusted for age and sex using quantile regression and for multiple comparison using Bonferroni correction.

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The correlation of clinical characteristics with antibody levels after the second vaccination was assessed jointly for participants with T1DM and T2DM. Age had an inverse and moderate to strong correlation with antibody levels ($r = –0.45$, $p < 0.001$), which was stronger for T1DM (–0.53, $p < 0.001$) than T2DM ($r = –0.20$, $p = 0.087$). eGFR had a positive and moderate correlation with antibody levels ($r = 0.28$, $p = 0.001$), BMI had an inverse and weak correlation ($r = –0.18$, $p = 0.027$), while HbA1c and glycaemic control had no significant correlation with antibody levels (176). See figure 4.5.2 for details.

The median antibody response was significantly higher ($p = 0.036$) in participants who had a body temperature >37.0 ºC after the second vaccination compared to those with a body temperature ≤37.0 ºC (176).

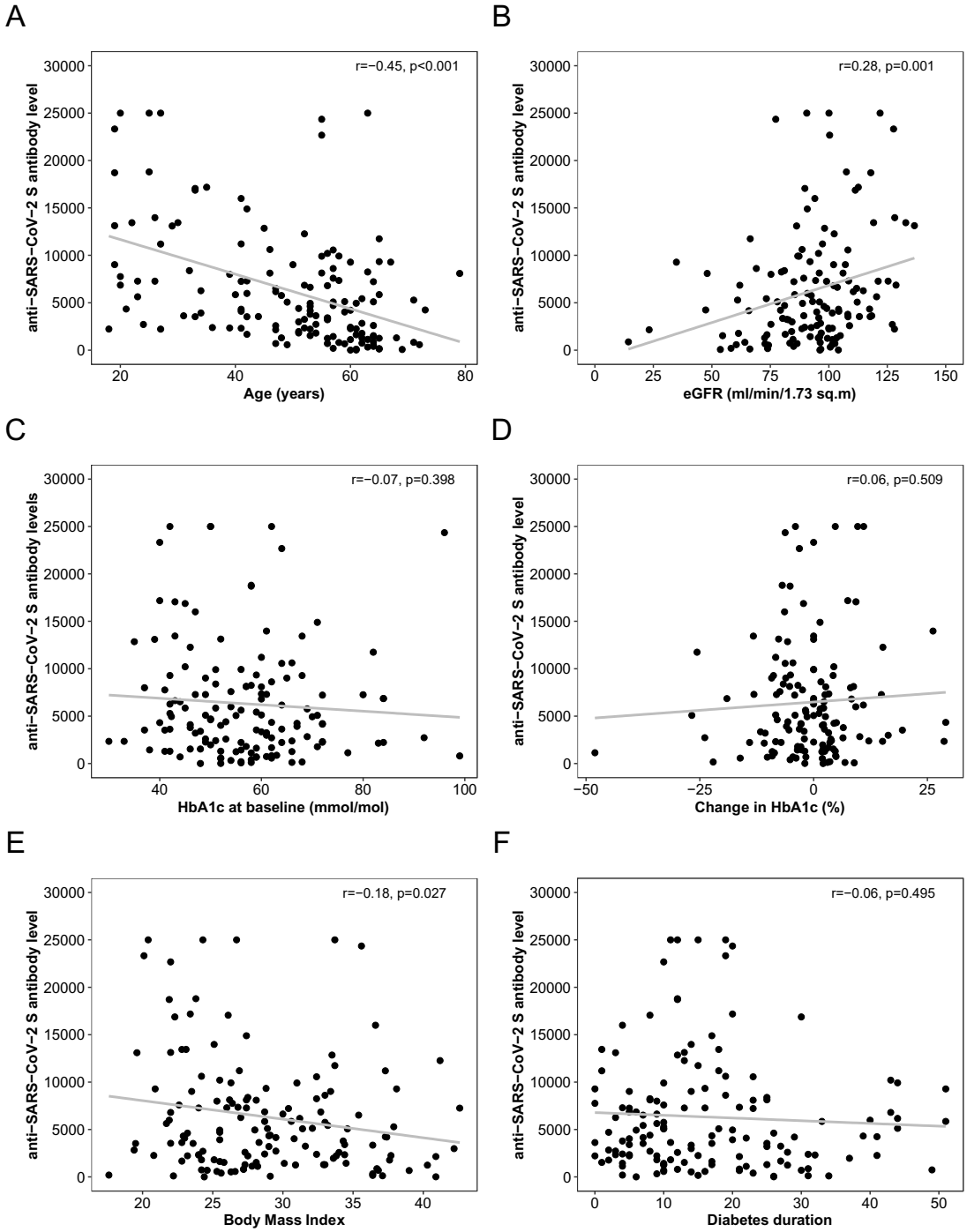

Figure 4.5.2. Correlation plots of anti-SARS-CoV-2 S antibody levels with selected clinical characteristics (176)**.**

eGFR: Estimated glomerular filtration rate, HbA1c: Glycated haemoglobin, P: P-value, r: Pearson's correlation coefficient. P-values are reported for Pearson's correlation test.

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4.6. Impact of COVID-19 vaccination on glycemia, insulin dosing pattern, and carbohydrate intake in people with diabetes mellitus

Of 161 participants enrolled in the COVAC-DM study, the CGM data were available for 74 participants to be included in this substudy. Of these participants, 78% had T1DM and 22% had T2DM (177).

In both T1DM and T2DM participants, no significant variations ($p = 0.962$, $p = 0.704$) were observed in the TIR over the period of vaccination (2 days before and 3 days after the vaccination). Similar trends were observed for TBR ($p = 0.952$, $p = 0.704$) and TAR ($p = 0.941$, $p = 0.715$) in T1DM and T2DM participants (177). See figure 4.6.1 for details.

Of all available CGM days, 35% were spent without any side effect (score = 0), 58% with at least one side effect (score = 1), and 7% with side effect score of 2. Based on the side effect score, participants with T1DM on average spent less TIR on days with a higher side effect score of 1 or 2 ($p = 0.045$). This finding was further confirmed with a higher TAR ($p = 0.040$) on days with side effect scores >0. Whereas the side effect score had no significant influence on TBR. In participants with T2DM, the side effect score had no significant influence on the TIR ($p = 0.865$), TAR ($p = 0.856$), and TBR ($p = 0.081$). While the side effect score had no significant influence on glycemic variability in both T1DM ($p = 0.206$) and T2DM ($p = 0.501$) participants (177). See figure 4.6.1 for details.

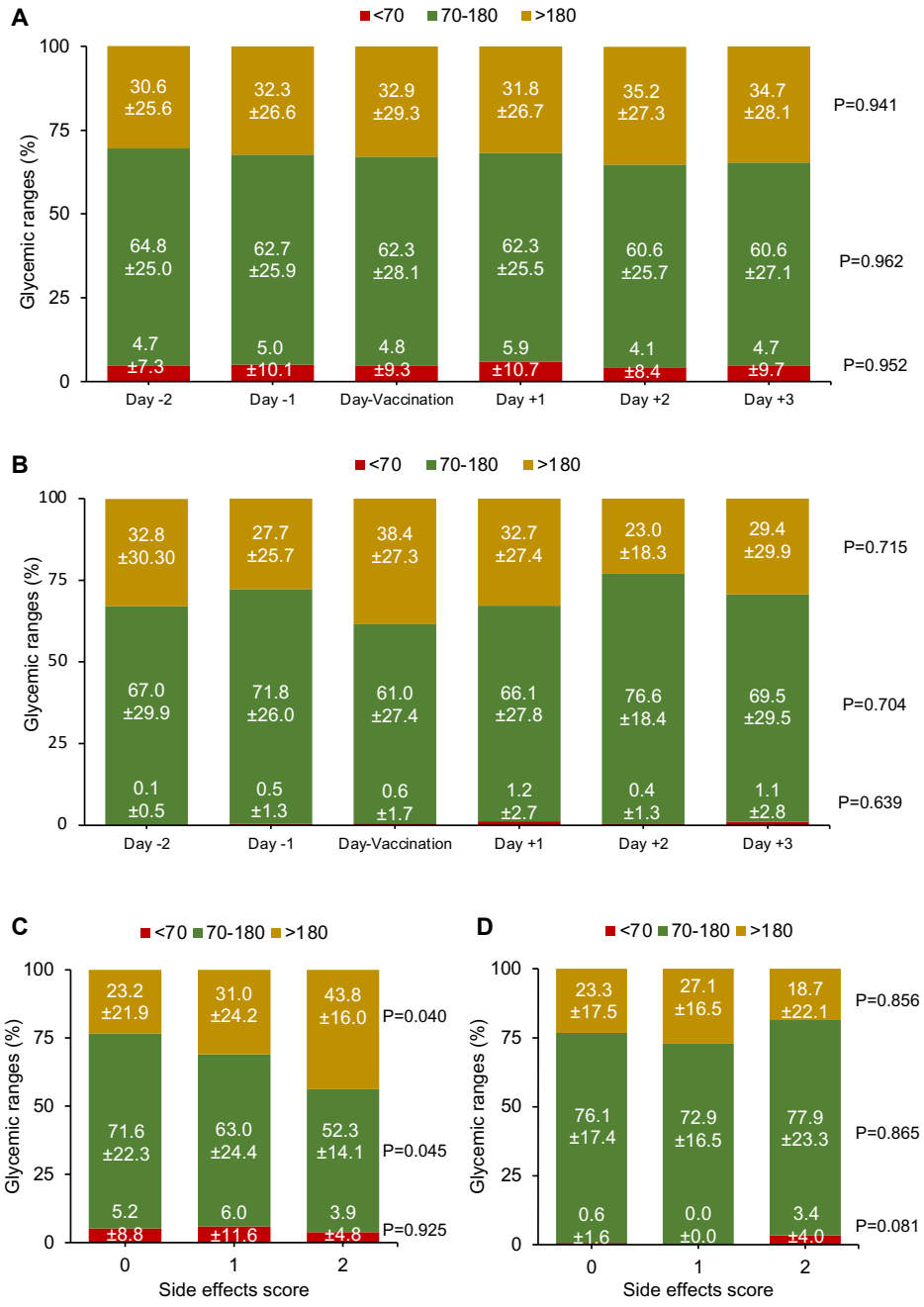

Figure 4.6.1. Glycaemic ranges in response to the COVID-19 vaccination in people with type 1 diabetes mellitus (A) and type 2 diabetes mellitus (B). Glycaemic ranges with respect to side effect score in people with type 1 diabetes mellitus (C) and type 2 diabetes mellitus (D) (177).

Glycemic ranges are given in mg/dL. P-values for glycaemic ranges in response to COVID-19 vaccination are reported for mixed linear model. P-values for side effects score are reported for one-way ANOVA.

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No adjustment in the insulin dosing was required during the vaccination period in participants with either T1DM (p = 0.578) or T2DM (p = 0.346). Likewise, carbohydrate intake did not differ significantly in participants with T1DM (p = 0.092) and T2DM (p = 0.958) around the COVID-19 vaccination (177). See figure 4.6.2 for details.

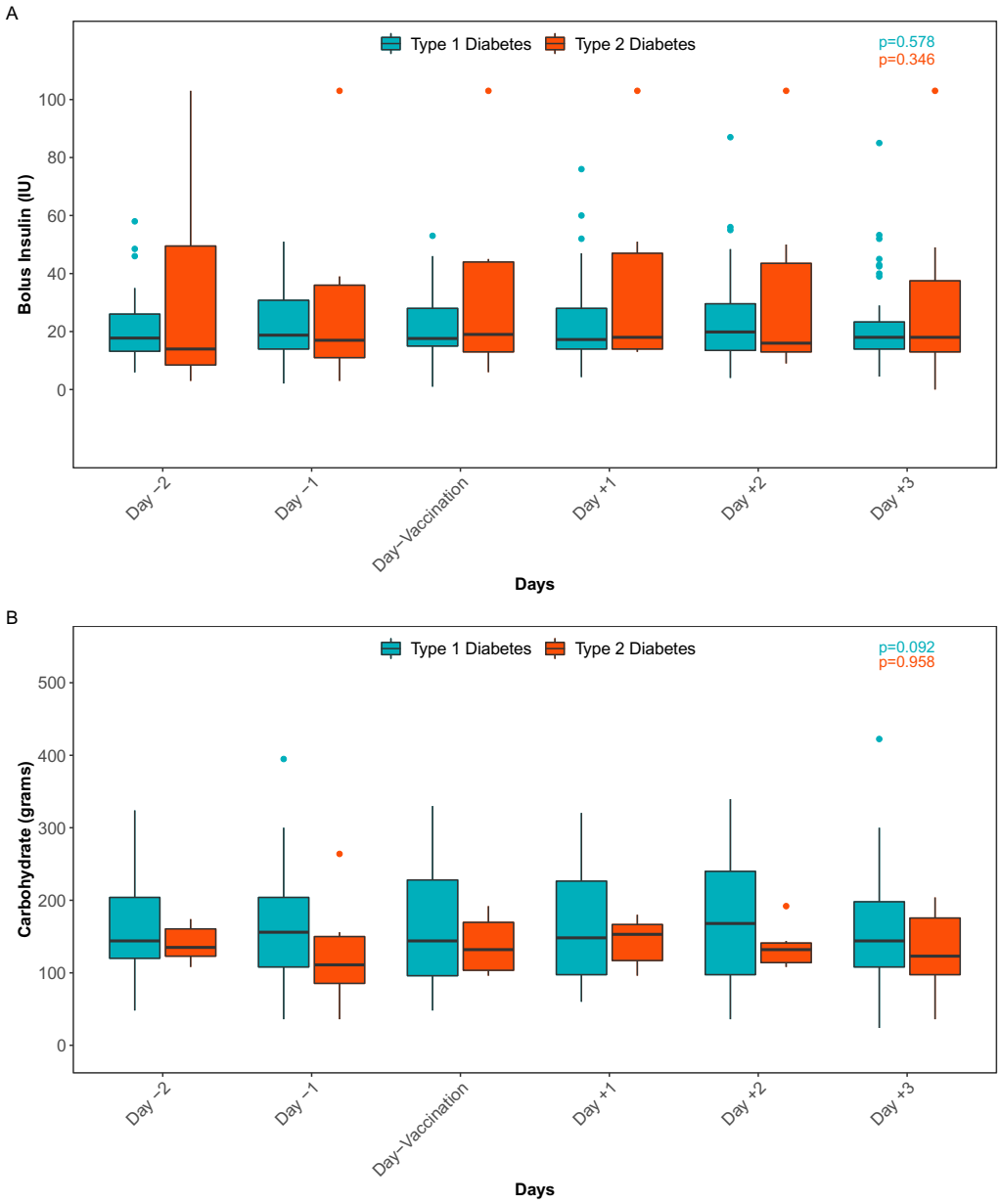

Figure 4.2.2. Bolus insulin dose and carbohydrate intake during vaccination period in people with type 1 and type 2 diabetes mellitus.

P-values are reported for repeated measures ANOVA.

Used with permission from the COVAC-DM study group.

5. DISCUSSION

5.1. In-hospital mortality in influenza patients with diabetes mellitus

In this retrospective analysis of the nationally representative AHI database, we investigated the characteristics specific to IRI hospitalization and significantly predicted subsequent short-term in-hospital mortality in people with diabetes. Our analysis revealed that the number of IRI-related hospitalizations peaked during the winter months in Austria. People hospitalized for IRI were more likely to be old, males, and had a higher individual and/or cumulative burden of comorbidities such as cancer, COPD, CHF, dementia, liver disease, and renal disease. The overall 30- and 90-day mortality rates were 8% and 10%, respectively. The risk of short-term mortality significantly increased with age, each-score point of ACCI, and in the presence of CHF and renal disease. Whereas influenza types, diabetes subtypes, and COPD had no significant influence on short-term mortality.

Of the total number of IRI-related hospitalizations between 2013 and 2017 across Austria, the highest number was hospitalized between November and February, while it was the lowest from May to October. This hospitalization trend for IRI in our cohort is similar to seasonal influenza incidence in temperate countries like Austria. As such, temperature countries experience seasonal epidemics of influenza in the winter months, increasing the hospitalization rate during this period (37).

In our cohort, patients hospitalized for IRI were more likely to be older than those hospitalized for other causes. Mainly, those over 70 years had 74–100% higher odds of being hospitalized for IRI. This finding is consistent with previous population-based studies from Canada and United States that showed an increasing trend in admission rates for influenza with increasing age (178,179). A meta-analysis of 234 studies also reported a 48% higher admission rate due to influenza-associated pneumonia in the elderly (65+ years) versus non-elderly (39). In addition to old age being a significant characteristic of IRI-related hospitalization in our cohort, both 30- and 90-day cumulative mortality rates increased with age, indicating that old age is a strong risk factor for IRI-related short-term mortality. For example, people aged 80+ had cumulative 30-day mortality of 11% and 90-day mortality of 15% compared to 2% each in those aged <50 years. This finding is consistent with a study from Spain, which reported mortality rates of 18% and 17% in hospitalized influenza patients aged 65–74 years and 75+ years, respectively, compared to 9% in those aged 18–64 years (40). Similarly, several other population-based studies from different countries demonstrated an excess

mortality risk following IRI in the elderly (39,179–182). Likewise, a meta-analysis assessing risk factors of influenza in the general population concluded that the odds of all-cause mortality were 2.95 greater in the elderly compared to non-elderly (39). These consistent findings from our cohort and previous studies confirm that age is an established risk factor for mortality in people with and without diabetes.

As most cases of influenza are self-limiting, hospitalization following the infection indicates a severe episode of illness. Our analysis showed that people with T1DM had 33% higher odds of hospitalization for IRI than those with T2DM. However, both 30- and 90-day mortality rates and corresponding adjusted hazard rates were not significantly different between types of diabetes. No comparable data on influenza hospitalization and mortality between types of diabetes are available, as most studies have not classified it into subtypes. Nevertheless, in a recent study from Norway, people with T2DM were 2.5 times more likely to be hospitalized with pandemic influenza compared with those without it, whereas the adjusted 90-day mortality ratio was lower in people with diabetes (aHR: 3.89, 95%CI: 1.25–12.06) than those without diabetes (aHR: 10.79, 95%CI: 7.23–16.10) (92). As diabetes is classified based on ICD-10 codes in the AHI database, misclassification of diagnoses is possible due to inaccurate coding, which might be a reason why mortality rates were similar between T1DM and T2DM in our study. Given the results of Norwegian and our study, hospitalization and mortality rates need to be interpreted with caution. Therefore, more studies are recommended to explore differences in influenza outcomes by subtypes of diabetes. Moreover, the AHI database does not capture information regarding diabetes duration or markers of glycemic control such as HbA1c. Therefore, we could not investigate the influence of these clinical parameters on hospitalization rates and associated mortality. According to an in vitro and in vivo animal study, alterations in blood glucose levels are correlated with a severe course of influenza (96). We speculate that several hospitalizations occurred due to dysglycaemia but not primarily due to influenza in our study, which was not captured in our analysis. Several studies support our speculation and have documented dysglycaemia as a significant risk factor for infectious diseases and mortality (183–185).

Our analysis revealed that in addition to age, comorbidities and their weighted cumulative burden measured as ACCI were important predictors for IRI-related hospitalization in people with diabetes. Specifically, people hospitalized for IRI were more likely to have cancer, COPD, CHF, renal disease, dementia, and a higher weighted cumulative burden of comorbidities compared to those hospitalized for other causes. Studies have not explicitly compared the individual or cumulative burden of comorbidities between influenza and non-influenza-related hospitalizations in people with diabetes. Nevertheless, our findings are

supported by a study from Mexico, in which 46% of patients hospitalized for influenza had at least one comorbidity, such as systemic arterial hypertension, obesity, diabetes, COPD, or CVD (186). Likewise, another study conducted during the influenza pandemic in Spain documented that more than 51% of hospitalized patients with influenza had a chronic disease such as asthma, diabetes, obesity, or cancer (187). While a countrywide study in France revealed COPD, heart failure, cirrhosis, and iron deficiency anaemia as common morbidities in patients hospitalized for influenza (188). However, in contrast to our study, none of these studies reported dementia and chronic renal disease as common comorbidities in hospitalized influenza patients or reported their association with hospitalization for influenza. Interestingly, a study comprising approximately six million elderly US citizens found that those with dementia had a lower burden of influenza infections and a shorter length of stay in the hospital compared to the general population (189). Regarding the role of cancer in developing severe influenza infection, it is challenging to determine whether cancer or its chemotherapy are the main culprits. It is noteworthy that the types of comorbidities associated with influenza hospitalization differ among studies. This disparity could be attributed to many factors, such as disease burden in the population, criteria for diagnosing and recording diseases, and lifestyle risk factors. Regardless, most studies point toward the strong individual or cumulative influence of these comorbidities on the severity of influenza infection.

In our diabetes cohort, comorbidities such as CHF and renal disease and the ACCI emerged as independent risk factors of 90-day mortality following hospitalization for IRI. The presence of CHF and renal disease elevated the risk of 90-day mortality by 97% and 50%, respectively. The excess mortality risk following influenza heart failure and renal disease patients is well documented. However, studies exploring the association of these diseases with mortality risk in IRI patients with diabetes are sparse. Nonetheless, in line with our findings, a meta-analysis showed that the presence of any CVD and CKD increased the odds of all-cause mortality by 192% and 211% in influenza patients, respectively. However, its results regarding renal disease were inconclusive due to a limited number of studies included in the meta-analysis (39). Similarly, a recent study based on the Indian national inpatient database found a significant association between influenza and heart failure in hospitalized patients (190). Concerning renal disease, a countrywide analysis of an inpatient database in the United States documented consistently higher age-adjusted in-hospital mortality in end-stage kidney patients and concluded that it independently increased the odds of mortality by 26%. In our cohort, as expected, the ACCI appeared as the strongest predictor of in-hospital mortality as an increase in this score by one unit elevated the risk of mortality by 14%. In line with our findings, a Spanish study reported ACCI as a significant predictor of fatality (191). These findings suggest

that the concurrent existence of certain chronic diseases substantially aggravates mortality risk following influenza by causing multi-organ failure during acute viral infections.

The findings of this study must be interpreted in the context of some limitations. In the AHI database, participating hospitals diagnose and record diseases as per ICD codes. Hence, there are chances that diseases were assigned incorrect ICD codes or even codes were not recorded for some diseases. Furthermore, information about the confirmatory test for influenza and its subtypes and severity was unavailable for all patients in the database. For the same anticipated issues in the ICD codes, diabetes was not classified into further subtypes. Therefore, findings concerning this population need to be interpreted with caution. The AHI database does not gather information about important laboratory and clinical parameters such as glucose, HbA1c, influenza vaccination status, previous IRI-related hospitalization, emergency care, and critical care admission. Thus, we could not evaluate the impact of these determinants on both hospitalization and mortality rates in our cohort. However, we believe that influenza vaccination status, which is ~6% in Austria (192), has no significant impact on our overall findings. This database only records the discharge date but not the admission date of patients. Therefore, we performed a time-to-event analysis using the discharge date, which might not reflect the actual time-to-mortality following the hospital admission. In this cohort, the number of people with type 1 diabetes was small, owing to its low prevalence in the country. Furthermore, our findings are limited to the diabetes population in Austria, as accessing nationally representative data from other European countries is a challenging and time-consuming procedure. Last, we could analyze patients hospitalized for IRI up to 2017 due to restrictions on using recent data for research purposes. Despite this limitation, we believe it is unlikely that the burden and pattern of risk factors and mortality of IRI would have changed significantly during the upcoming years.

To conclude, our retrospective analysis of the countrywide AHI database showed that people with diabetes hospitalized for IRI were more likely to be old and suffer from multiple morbidities as compared with those hospitalized for other causes. Furthermore, among those hospitalized following IRI, in addition to age and individual morbidities, the weighted cumulative score of comorbidities called ACCI emerged as the strongest predictor of short-term mortality. Therefore, this tool could be utilized to identify people at high risk for hospitalization and subsequent mortality. Last, our analysis provided real-world evidence that people with diabetes may have a varying risk of experiencing severe IRI and subsequent fatality that is attributable to age and the burden of comorbidities. These findings can inform healthcare providers and stakeholders in identifying high-risk people within the diabetes population to guide clinical management and vaccination policy for IRI.

5.2. Adverse outcomes of COVID-19 in hospitalized patients with diabetes mellitus

In this retrospective cohort study of the nationally representative GÖG database, we evaluated the association of diabetes with the severity of infection (measured as admission to ICU) and in-hospital mortality in patients hospitalized for COVID-19. We found that hospitalized COVID-19 patients with diabetes were more likely to be old and had a higher prevalence of multimorbidity and the ACCI score than those without diabetes. The PSM analysis, matched for characteristics, comorbidities, and ACCI score, showed that diabetes was significantly associated with increased odds of ICU admission, whereas it was not significantly associated with in-hospital mortality. In addition, old age, being male, and comorbidities such as myocardial infarction, CHF, COPD, pulmonary circulation disorders, neurological disorders, renal disease, coagulation disorders, and the ACCI score were associated with increased odds of ICU admission and in-hospital mortality.

In the unmatched cohort, diabetes was associated with 24% increased odds of in-hospital mortality. The magnitude of this association decreased three times from 24% to 8% in the PSM cohort and became insignificant. These results indicate that diabetes has no independent influence on in-hospital mortality whatsoever, and the observed association is attributable to old age, sex, and various comorbidities. Our results align with a previous study that also performed the PSM analysis on French COVID-19 patients. According to that study, diabetes had no significant impact on ICU admission or in-hospital mortality (HR: 1.29, 95%CI: 0.95–1.41, p = 0.140) (128). Similarly, another cohort study revealed that diabetes was no longer significantly associated with in-hospital mortality after adjusting for age, sex, and several major comorbidities such as CVD, hypertension, and chronic respiratory diseases, among others (127). While our results are contrary to the vast majority of studies that have reported diabetes as a significant risk factor for in-hospital mortality in COVID-19 patients. In this perspective, according to a recent meta-analysis, diabetes significantly increased the odds of in-hospital mortality by 85%, which decreased after accounting for age, sex, and comorbidities; however, the association remained significant (193). Likewise, diabetes was associated with 121% higher odds of mortality in COVID-19 patients (194). Another large study from England concluded that diabetes increased the odds of death by 80% (195). Similarly, two studies conducted in China showed that diabetes was a significant predictor of mortality in COVID-19 patients, and the adjustment for multiple chronic diseases and laboratory parameters did not change this relationship (196,197). Although many studies have shown diabetes as a significant risk factor for COVID-19 mortality, most of these studies and even ones included in

meta-analyses have not accounted for many risk factors and comorbidities, thereby raising concerns over the implications of their findings. Moreover, a recent study that applied the PSM method to compare in-hospital mortality before and after the COVID-19 pandemic found underlying severe medical conditions as significant contributors to mortality (198). Moreover, these limitations provide further sustenance to the significant role of age, sex, and comorbidities on COVID-19 mortality in patients with diabetes.

In the unmatched cohort, the odds of severe COVID-19 infection, measured as ICU admission, were 36% higher for patients with diabetes than those without diabetes. Although these odds decreased by more than twice (15%) in the PSM cohort, the association remained significant. These results are in line with the previously published research. However, it is difficult to directly compare the results across studies because of a vast discrepancy in the defining criteria of COVID-19 disease severity. Also, the strength of association between diabetes and disease severity is much lower in our study compared to most of the previous studies. As such, according to four meta-analyses conducted in various phases of the COVID-19 pandemic, the pooled odds ratios of severe COVID-19 disease for diabetes varied from 1.58 to 2.75 (50,199–201). Such drastic heterogeneity in the magnitude of association across meta-analyses existed because included studies did not uniformly adjust for confounding factors such as age, smoking status, obesity, and various underlying chronic conditions (50,200). Furthermore, according to a previous study, patients using insulin had 258% higher odds of severe COVID-19 illness compared to those not using insulin (202). However, adjustment for glucose-lowering medicines was not possible in our study due to the unavailability of this information in the GÖG database.

Our study has some limitations related to the GÖG database. We did not classify diabetes into its subtypes and perform any subgroup analysis due to the chances of incorrect ICD coding or miscoding of diseases. In addition, this database did not capture data regarding glucose measurement, HbA1c, pharmacotherapy, and other laboratory parameters for all patients. Therefore, we did not balance these variables in our PSM analysis. Due to this limitation, residual confounding may still exist between diabetes and adverse COVID-19 outcomes. Moreover, in this database, in-hospital mortality was defined as death occurring due to any underlying cause. According to this definition, in-hospital mortality could have also included deaths from causes other than COVID-19. Nevertheless, as this database only captures data of COVID-19 patients, the chances of including deaths from other causes are minimal. Another limitation is that we could not analyse the long-term outcomes of COVID-19 in people with diabetes. Finally, the analysis was performed on Austrian patients only; therefore, its findings may not be transferable to other populations.

In conclusion, our analysis of hospitalized COVID-19 patients enrolled in the GÖG database showed that people with diabetes were more likely to be old and had a higher prevalence of various comorbidities compared to those without diabetes. In line with the previous literature, people with diabetes had a higher likelihood of experiencing severe COVID-19 than those without diabetes. While in contrast with the majority of existing evidence, diabetes was not found to be an independent risk factor for in-hospital mortality. Instead, advanced age and comorbidities were identified as underlying reasons for the increased odds of in-hospital mortality in COVID-19 patients. Our robust analytical method of PSM established that both age and multimorbidity significantly contribute to the development of adverse outcomes within diabetes. Therefore, we recommend that healthcare professionals and stakeholders consider these risk factors for risk estimation, clinical management, and national vaccination campaigns.

5.3. Biomarkers of in-hospital mortality in COVID-19 patients with diabetes mellitus

This retrospective analysis of the multicenter COVID-19 in diabetes registry investigated the predictive role of several inflammatory (LDH, CRP, IL-6, PCT, Ferritin), hepatic (ALT, AST, ALT–AST ratio), coagulation (D-dimer), and cardiac biomarkers (Nt-proBNP, Troponin T) in hospitalized COVID-19 patients with established diabetes and prediabetes. T2DM was present in 71% of hospitalized patients, T1DM in 6%, and prediabetes in 15% of patients. In-hospital mortality occurred in 19% of hospitalized patients. Among studied biomarkers, CRP, PCT, IL-6, AST–ALT ratio, D-dimer, Nt-proBNP, and Troponin T were found significantly elevated in non-survivors versus survivors as well as independently associated with in-hospital mortality. Of the significant biomarkers, only cardiac biomarkers such as NT-proBNP and Troponin T showed good discrimination of 0.74 and 0.81, respectively. NT-proBNP showed good calibration, while Troponin T showed poor calibration.

In our cohort, in-hospital mortality occurred in 19% of COVID-19 patients with prediabetes and diabetes. This observed mortality rate is comparable with a cross-sectional study that reported a mortality rate of 20% in Korean COVID-19 patients with diabetes versus 5% in those without diabetes (203). In contrast, a small single-center study showed a mortality rate of 38%, which was two times higher than our mortality rate (204). While our previous analysis of the nationally representative GÖG database revealed a slightly lower mortality rate of 14% in people with diabetes (173). Similarly, a systematic review and meta-analysis of 145

studies reported that diabetes increased the absolute risk of death in COVID-19 patients by 14% (205). However, it is challenging to compare mortality estimates for COVID-19 due to a complex relationship of population, healthcare, and patient-related factors with diabetes and mortality.

In our analysis, the cardiac biomarker Troponin T was found significantly elevated in COVID-19 patients with prediabetes and diabetes who died versus those who survived. Also, increased levels of Troponin T were significantly associated with 120% increased odds of in-hospital mortality. Troponin T is a biomarker for myocardial damage and its elevated levels indicate acute cardiac injury (51). It has been established that SARS-CoV-2 infection can cause cardiac injury via various pathophysiological mechanisms such as cytokine storm, increased inflammatory response, disturbances in coagulation pathways, increased myocardial stress, hypoxia, and direct damage to cardiomyocytes (51,52). The role of Troponin T in COVID-19-induced cardiac injury and subsequent mortality has also been frequently described in clinical and epidemiological studies. For instance, according to a recent meta-analysis, an acute cardiac injury occurred in 5-38% of COVID-19 patients, with an overall rate of 21%. Moreover, the severity and prognosis of COVID-19 were found to be significantly associated with the degree of elevation in Troponin levels (206). Interestingly, COVID-19 mortality was significantly influenced by the presence of underlying CVD and levels of Troponin. As such, according to a hospital-based study from China, patients with existing CVD had higher Troponin T levels than those without CVD (55% vs. 13%) (207). Similarly, another study reported a higher prevalence of CVD in COVID-19 patients with increased levels of Troponin I (208). Furthermore, the in-hospital mortality was 8% in patients without existing CVD and normal Troponin T levels, 13% in those with existing CVD and normal Troponin T levels, 37% in those without CVD but elevated Troponin T levels, and 69% in those with existing CVD but elevated Troponin T levels (207). While a large cohort study of COVID-19 patients demonstrated that an increase in troponins from undetectable to high levels was associated with increased odds of death from 68% to 241% (209). The findings of all these studies suggest that COVID-19 frequently causes cardiac injury, which subsequently increases the risk of mortality. Moreover, patients with existing CVD are at a higher risk of COVID-19-induced cardiac injury and its related death compared to those without CVD. In addition to these studies, many narrative and systematic reviews have also recommended measuring cardiac troponins as a part of routine clinical assessment in COVID-19 patients for the timely diagnosis of cardiac injury and evaluation of prognosis (206,210,211).

Like Troponin T, NT-proBNP was significantly elevated in patients who died versus those who survived in our cohort. In addition, an increase in NT-proBNP levels was associated

with 50% higher odds of mortality. As NT-proBNP is a biomarker of hemodynamic stress and cardiovascular dysfunction, its increased levels might indicate the worsening of CHF in COVID-19 patients irrespective of the infection (211). However, NT-proBNP has also been found elevated in patients with severe respiratory illnesses in the absence of CHF. In COVID-19 patients, NT-proBNP levels could also be disrupted due to myocardial dysfunction caused by the infection. Various pathophysiological mechanisms such as cytokine storm, COVID-19-induced cardiac damage, myocardial wall stress, and hypoxia have been postulated to be responsible for the abnormal release of NT-proBNP in COVID-19 infection (51,52,211). Our findings coincide with a meta-analysis of 12 studies that reported significantly higher pooled mean NT-proBNP levels of 790.57 pg/ml in patients who died or had severe COVID-19 disease compared to 160.56 pg/ml in patients who were alive or had non-severe disease (212). Likewise, another meta-analysis revealed that natriuretic peptide levels were significantly higher in patients who died or had severe disease than in those with mild disease or who were alive (213). Similarly, a study from China comprising 28 patients with diabetes and COVID-19 also reported that NT-proBNP was significantly increased in ICU patients compared to non-ICU patients (214).

Studies have also assessed the association of NT-proBNP with in-hospital mortality. In this regard, a meta-analysis comprising six studies concluded that NT-proBNP elevation increased the pooled adjusted mortality risk by 37% in COVID-19 patients (215). A recent study reported that elevation in levels of NT-proBNP increased the risk of mortality by 28% (216). Likewise, a small study in severe cases of COVID-19 in China reported that a change in NT-proBNP of 100 pg/ml was associated with an increased risk of mortality by 20–28% (217). We also assessed the association between NT-proBNP and mortality by excluding a cohort of patients with existing CHF. Interestingly, the magnitude of association between NT-proBNP and mortality became stronger after excluding CHF patients, indicating the independent influence of NT-proBNP on mortality in COVID-19. Similar to our analysis, a study of patients with severe COVID-19 pneumonia without existing heart failure found that NT-proBNP was associated with a 115% higher risk of mortality (218). Another study among Egyptian COVID-19 patients showed that the risk of mortality was 3.41 times higher in people with NT-proBNP levels of 331 pg/ml and above and 3.84 in those with NT-proBNP levels of 11,126 pg/ml and above compared to the patients with its lowest levels (219). Also, a large cohort study of COVID-19 patients demonstrated that an increase in NT-proBNP from the lowest to the highest level was associated with 47% to 174% increased odds of death (209). In the light of this compelling evidence regarding the impact of NT-proBNP on disease severity and mortality of COVID-19, recent reviews and editorials have also recommended measuring NT-proBNP

routinely for the evaluation of disease severity and prognosis of COVID-19 patients (210,211). Although the association of cardiac biomarkers with COVID-19 outcomes has been thoroughly investigated, a few studies have evaluated the predictive performance of these cardiac biomarkers. According to a recent small meta-analysis of six studies, the pooled discrimination of included studies was 0.90 for NT-proBNP (215), which is significantly higher than the discrimination observed in our study.

Although the prognostic role of various cardiac biomarkers in COVID-19 patients has been well researched, clinical and epidemiological data in patients with diabetes are scarce. Our study attempted to address this gap and showed that, like the general population, the cardiac biomarkers Troponin T and NT-proBNP were significantly associated with mortality and showed good discriminatory performance of 0.81 and 0.74, respectively, in diabetes patients. Unfortunately, we could not compare the predictive performance of these biomarkers with non-diabetes patients due to the unavailability of data for non-diabetes COVID-19 patients. It is crucial to evaluate the predictive role of cardiac biomarkers in COVID-19 patients with coexisting diabetes for several reasons. Diabetes frequently coexists with various CVDs (220). Also, people with diabetes often have asymptomatic coronary artery disease and heart failure (221). Both symptomatic and asymptomatic CVDs in diabetes increase the risk of acute coronary syndrome, cardiac injury, heart failure, and arrhythmias during COVID-19 infection due to direct myocardial damage, cytokine storm, and hypercoagulation caused by the SARS-CoV-2 virus (104,211). In addition, hypoglycaemia and drugs administered for treating COVID-19 may also induce arrhythmias (222,223). All these underlying diabetes- and COVID-19-related factors may be responsible for altering cardiac biomarkers in people with diabetes. Therefore, it is challenging in this population to establish whether these biomarkers are altered due to COVID-19 infection or other contributing factors. In our study, we eliminated the influence of some clinical factors and existing CHF on the association between cardiac biomarkers and COVID-19 mortality; however, we could not control for all factors. Given these reasons, the role of both Troponin T and NT-proBNP must be evaluated with caution and in relation with both existing and incident CVDs in COVID-19 patients with diabetes. We also recommend more studies comparing the role of these cardiac biomarkers between diabetes and non-diabetes COVID-19 patients.

Among inflammatory biomarkers, CRP, LDH, IL-6, and PCT, except ferritin, were significantly associated with in-hospital mortality in our cohort. However, none of these biomarkers showed good predictive performance. Although the prognostic role of inflammatory markers has been thoroughly explored in COVID-19, such studies are scarce in people with diabetes. In addition, the published studies in this patient population have inadequate sample

sizes or did not evaluate the predictive performance of these biomarkers. As such, a retrospective study from India investigated various biomarkers in severely ill COVID-19 patients with and without diabetes. In this study, the CRP was significantly associated with increased COVID-19 mortality in 57 diabetes patients, with an AUC of 72%, which is 10% higher than the AUC in our study (224). While another small study comparing 28 COVID-19 patients with diabetes from China reported that the concentrations of LDH, PCT, CRP, ferritin, and IL-6 were significantly elevated in patients admitted to ICUs than in those not admitted to ICUs (214). However, the predictive performance was not determined for any of these biomarkers in this study. In contrast, a study of 118 severe COVID-19 patients with diabetes documented that LDH and CRP levels were significantly elevated; however, both were not significantly associated with mortality (204). On the other hand, a cross-sectional analysis of 103 patients with prediabetes and diabetes from the United Arab Emirates showed significantly higher levels of CRP (137 ±112 vs. 34 ±39) and ferritin (1762 ±2586 vs. 358 ±442) in patients who required ICU care versus those who required ward care (225). Given that most existing studies have not explored the role of inflammatory biomarkers in COVID-19 patients with diabetes, we recommend more studies in larger diabetes cohorts.

In COVID-19 patients with diabetes, inflammatory biomarkers can be disrupted due to various reasons. Diabetes is characterized by chronic low-grade inflammation and a dysfunctional immune system due to chronic hyperglycaemia, which elevates the levels of various inflammatory biomarkers such as cytokines and CRP (104,115). This chronic hyper-inflammatory state induces an exaggerated immune response to COVID-19 infection, resulting in a more pronounced rise of inflammatory biomarkers in diabetes compared to non-diabetes. In addition, abrupt hyperglycaemia can also be developed from COVID-19 due to changes in food intake and absorption, steroid therapy, adjuvant drug therapy, cytokine storm, and dysfunctional immune response. These COVID-19-related abrupt glycaemic changes may also increase cytokine and CRP levels in people with diabetes (115). Unfortunately, we could not compare the predictive performance of biomarkers between diabetes and non-diabetes patients in our cohort due to the unavailability of data for non-diabetes patients. Notwithstanding, a few clinical studies have shown that various inflammatory and immune system-related biomarkers such as neutrophils, ferritin, cytokines, and CRP are substantially more elevated in COVID-19 patients with diabetes compared to non-diabetes (60,226). Likewise, a meta-analysis reported significantly higher levels of serum ferritin (standardized mean difference [SMD]: 0.47, $p = 0.002$), CRP (SMD: 0.53, $p = 0.002$), and IL-6 (SMD: 0.31, $p = 0.005$) in COVID-19 patients with diabetes than those without diabetes (227). While another study has confirmed the partial mediatory role of CRP between diabetes and severe COVID-

19 infection, confirming the crucial role of inflammation in the pathogenesis of COVID-19 in diabetes (228). Although evidence strongly suggests a severe inflammatory response to COVID-19 infection in patients with diabetes than those without diabetes, studies need to compare the predictive performance of these biomarkers between diabetes and non-diabetes patients. Besides, the complex immune and inflammatory processes present a serious challenge in investigating the independent impact of inflammatory biomarkers on adverse COVID-19 outcomes in people with diabetes. Therefore, disruptions in inflammatory biomarkers following COVID-19 in this patient population should be interpreted with caution and probably should be correlated with other biomarkers and clinical factors for better decision-making.

In our cohort, D-dimer, a biomarker for activated fibrinolysis, was found significantly elevated in patients who died compared to those who were discharged alive. However, it was not significantly associated with mortality in the adjusted analysis and demonstrated a below average discriminatory performance, having an AUC of 66%. Compared to our study, a retrospective study from Morocco found that D-dimer levels were significantly more elevated (1745 vs. 845, $p < 0.001$) in COVID-19 patients with diabetes compared to non-diabetes, with an AUC of 75% (229). Another small study (N = 28) from China also found D-dimer levels significantly elevated in diabetes patients with severe COVID-19 infection versus those without severe infection (214). Another study conducted in the United Arab Emirates reported that COVID-19 patients with prediabetes and diabetes requiring ICU care had a higher mean concentration of D-dimer (2.3 ±3.5 µg/ml) as compared to those not requiring ICU care (0.7 ±0.5 µg/ml) (225). However, the predictive performance of D-dimer was not investigated by these studies. A few studies have also compared the levels of D-dimer between diabetes and non-diabetes COVID-19 patients. According to a study from China, the median levels of D-dimer were significantly higher (2.6 µg/ml vs. 1.2 µg/ml, $p = 0.012$) in hospitalized COVID-19 with diabetes compared to those without it. Also, within diabetes patients, median D-dimer levels were found significantly elevated in those who died (4.95 µg/ml vs. 0.41 µg/ml, $p < 0.001$) versus those who were alive (226). Likewise, another study reported a higher mean level of D-dimer (981 vs. 791, $p = 0.001$) in diabetes compared to non-diabetes COVID-19 patients (230). Furthermore, a meta-analysis showed significantly higher levels of D-dimer (SMD: 0.54, $p = 0.005$) in COVID-19 patients with diabetes than in non-diabetes (227). Although we could not compare the levels of D-dimer with non-diabetes patients, these consistent findings of studies worldwide confirm that COVID-19 patients with diabetes are more susceptible to coagulation dysfunction.

Like cardiac and inflammatory biomarkers, it is challenging to evaluate the exact role of coagulation biomarkers such as D-dimer in COVID-19 patients with diabetes. Diabetes is known to increase the risk of atherothrombotic events via various pathways. Hyperglycaemia in diabetes induces oxidative stress, which can lead to thrombin production. Also, persistent hyperglycaemia can cause inflammation and endothelial dysfunction, which leads to thrombus formation. Moreover, transient hyperglycaemia induced by oxidative stress and COVID-19 infection can disrupt the coagulation cascade, eventually leading to atherothrombotic events. Another pathway is the direct activation of thrombin and monocytes mediated by severe hypoxia (231). Research also shows that both chronic and acute hyperglycaemia exert similar effects on the coagulation pathways. In addition, both chronic and acute hyperglycaemia frequently result in hyperinsulinemia, which has also been found to produce prothrombotic events (232). These factors altogether lead to thrombosis and hypercoagulation in diabetes, which can be assessed by measuring levels of D-dimers.

Among hepatic biomarkers, we only evaluated the role of the AST–ALT ratio in our study. Like inflammatory and coagulation biomarkers, the AST–ALT ratio was significantly associated with mortality; however, its predictive performance was unsatisfactory (AUC: 0.65). In comparison, a study found no difference in hepatic enzymes such as ALT and AST between diabetes and non-diabetes COVID-19 patients; however, AST was significantly higher in patients who died compared to those who survived (226). According to another study, elevated ALT levels were significantly associated with mortality in COVID-19 patients with diabetes (204). Elevation of liver enzymes suggests an acute liver injury caused by the SARS-CoV-2 infection via various mechanisms. As biliary and hepatic endothelial cells in the liver contain ACE2 receptors, which provide a site for the replication of the SARS-CoV-2 virus and hence can directly damage liver cells. The liver injury could also result from an inflammatory response mediated by the immune system in response to the SARS-CoV-2 infection. Another cause of liver injury unrelated to COVID-19 can be associated with hepatotoxic medications such as antivirals and antibiotics used for treating COVID-19 (233).

This study had many strengths. One strength was its adequate sample size, which provided us with sufficient power to investigate the predictive role of various biomarkers in COVID-19. Another strength was the longitudinal study design of this study, which allowed us to prospectively ascertain the impact of each biomarker measured at the time of admission on the subsequent mortality. As data were collected from various hospitals in Austria, our findings can be extrapolated to the entire country. The availability of detailed information about the medical history and characteristics of patients enabled us to adjust for known factors that could confound the influence of biomarkers on mortality in COVID-19 patients.

This study encountered some limitations as well. The major limitation was the unavailability of data for COVID-19 patients without diabetes to perform a comparative analysis. Moreover, we could not perform the sub-group analysis based on types of diabetes due to an inadequate sample size for all subtypes. Next, since this study employed a pragmatic study design to collect data, the subtyping of diabetes might not be correct. Moreover, biomarker data were collected from routine laboratory measurements of each participating hospital, therefore, measurements were unavailable for all patients. Also, due to the pragmatic nature of this study, data for various previously identified biomarkers such as Troponin I, creatinine kinases, neutrophil to lymphocyte ratio, and cytokines were not collected and therefore could not be evaluated in patients with diabetes. Last, all biomarkers were measured only at the time of admission in each patient; their single measurement may not provide the entire picture of the clinical course and prognosis of COVID-19.

Based on our findings, cardiac biomarkers NT-proBNP and Troponin T emerged as significant predictors of short-term mortality in COVID-19 patients with established prediabetes and diabetes. Since it is feasible to measure circulating levels of NT-proBNP and Troponin T in any laboratory setting; we recommend measuring these biomarkers as a part of routine laboratory workup for the timely diagnosis of cardiac injury, assessment of cardiac dysfunction and disease severity, and evaluation of prognosis of COVID-19 patients. We also recommend more research focusing on the diagnostic and prognostic role of all major cardiac biomarkers in large cohorts of patients with diabetes. In addition, future studies need to explore if NT-proBNP and Troponin T should be incorporated into predictive tools to improve risk stratification and guide the clinical management of COVID-19 patients. Furthermore, comparing the predictive performance of NT-proBNP between patients with and without diabetes in future studies may provide valuable insight into its clinical significance in these patients. Additionally, investigating the relationship of repeated measurements of NT-proBNP with adverse COVID-19 outcomes may provide a better understanding of the usefulness of the trajectories of NT-proBNP to evaluate the treatment response and prognosis in this patient cohort.

5.4. Performance of SAPS 3 in critically ill COVID-19 patients with and without diabetes mellitus

In this retrospective analysis of the nationally representative GÖG database, we used global, Central European, and Austrian (calibrated in COVID-19 patients) versions of SAPS 3

to predict in-hospital mortality in critically ill COVID-19 patients. We also compared the predictive performance of these equations between patients with and without diabetes. Both global and Central European equations under-predicted the in-hospital mortality in three studied cohorts. In comparison, the Austrian equation, calibrated for COVID-19 patients, demonstrated adequate predictive performance in all cohorts. Concerning the predictive performance of SAPS 3, all equations showed low discrimination in all three cohorts. Also, the discriminatory performance of all equations was similar between patients with and without diabetes. Concerning the forecasting power of SAPS 3, the value of the brier score was >0.20 for all versions, indicating the low forecasting ability of the tool. The calibration of both global and Central European equations was unsatisfactory in all patient cohorts, particularly in patients without diabetes mellitus. Although the Austrian equation exhibited the best calibration among all SAPS 3 equations, the calibration was only adequate for the diabetes cohort.

In our study, both uncalibrated versions of SAPS 3 underpredicted the in-hospital mortality, and the discrimination of both uncalibrated and calibrated versions of SAPS 3 was unsatisfactory, as indicated by the AUC of 69% each. In addition, the forecasting power was poor, as indicated by the Brier score >0.20 for each equation. Many studies have evaluated the performance of various prognostication scores in critically ill COVID-19 patients (69,234,235). However, despite the SAPS 3 being a commonly used prognostication tool, a few studies have evaluated its performance in COVID-19 patients. In this regard, a study conducted in three ICUs in the United Kingdom showed that the SAPS II, a previous version of SAPS 3, underestimated the risk of mortality and inaccurately classified patients in risk strata (235). In contrast to our study, a validation study conducted in the earlier phase of the COVID-19 pandemic showed that the SAPS 3 had satisfactory discrimination with the AUC of 75% in 1464 Austrian COVID-19 patients. Although the discrimination was satisfactory in that study, the in-hospital mortality was significantly underpredicted, as indicated by the SMR value of 1.20. This underprediction was more obvious in low-risk strata of patients, which questions the clinical utility of this tool in COVID-19 patients (72). Similarly, another validation study of the regional SAPS 3 equation in Brazilian COVID-19 patients reported excellent discrimination of 83% and an SMR value of 0.95 (71). Likewise, a study carried out in the northeast region of Brazil evaluated the performance of SAPS 3 in COVID-19 patients admitted to ICUs during the first and second waves of COVID-19. According to this study, the SAPS 3 score exhibited excellent discrimination (AUC = 90%) in the first wave, while lower yet good discrimination (AUC = 81%) in the second wave of the pandemic (73).

As discussed above, existing studies have reported stark differences in the discriminatory performance of SAPS 3 in COVID-19 patients. We contemplate that several

endogenous and exogenous factors could have contributed to the inconsistent performance of this tool. Our cohort comprised COVID-19 patients from earlier and later waves of the COVID-19 pandemic; therefore, significant changes in treatment regimens and clinical management would have occurred during these waves of the pandemic. The recent Brazilian study supports our assumption as it showed a vast difference in the performance of SAPS 3 in the first and second waves of the COVID-19 pandemic (73). The developers of SAPS 3 also acknowledge the impact of therapeutic advancements on the performance of this tool (70). Other contributing factors could be related to healthcare infrastructure and resources, competency of healthcare professionals, and treatment protocols, among others. The burden and severity of population-specific factors such as smoking status, obesity, age distribution, multimorbidity, and disease severity could also impact the prognosis of COVID-19 (236). Many of these risk factors are not considered in the risk equation of SAPS 3, which could be a reason for notable differences in the discrimination of this score across studies. Among the endogenous factors, the SAPS 3 score is calculated based on the presence and magnitude of certain risk factors, which is central to the discrimination of this tool (237). Moreover, this tool does not include age and measurements of physiological disturbances as continuous parameters in its risk equation. Instead, it categorizes these parameters before including them in the risk equation. This approach could compromise the tool's ability to accurately estimate each measurement's impact on the prediction of the outcome (70,238). This particular approach adopted by the SAPS 3 has also been criticized by a multicenter European study (239). Last, several chronic diseases such as CVD, COPD, and renal disease, and some biomarkers related to inflammatory, coagulation, and cardiovascular system have been shown to increase the risk of severe COVID-19 disease and its mortality (240). Notwithstanding, unlike COVID-19-specific prognostication scores (62,241), many of these risk factors and biomarkers are not considered in SAPS 3 scoring, which could have compromised the predictive power of SAPS 3 in COVID-19.

The performance of any prediction score is measured in terms of discrimination and calibration. Discrimination means how well the tool can identify people who will develop the outcome. Calibration refers to an agreement between observed and predicted outcomes in different risk strata (237). If a score yields a good discriminatory performance, it does not necessarily ensure that that score will also yield a good calibration. As patients suffering from COVID-19 face a high risk of developing severe disease and mortality, well-calibrated prognostication tools are vital for accurate risk stratification and thereafter clinical management of patients. However, our analysis revealed a significant miscalibration in global and Central European equations of SAPS 3, especially in low-risk and medium-risk strata of patients. Our

findings are consistent with previous studies that assessed the performance of SAPS 3 in COVID-19 patients. For instance, a study performed on Brazilian COVID-19 patients reported that the SAPS 3 was miscalibrated in high-risk strata. While miscalibration was more evident in low-risk strata in Austrian COVID-19 patients, which was also the same in our analysis (71,72). Many studies have raised concerns about the calibration of SAPS 3 in various patient populations. In this regard, a systematic review of 28 studies concluded that the issue of miscalibration in the SAPS 3 score was evident in 57% of included studies (242). In addition, a large validation study of 50,000 ICU patients in Brazil found that despite satisfactory discrimination, the SAPS 3 version for Central and South America overpredicted mortality in patients and was significantly miscalibrated (243). Similarly, a multicenter study from 17 European countries found that the SAPS 3 overestimated the mortality, particularly in high-risk strata of patients, as indicated by SMR values of 0.75 and 0.91, respectively (239). All the evidence from various patient populations, including COVID-19, suggests that SAPS 3 is prone to misclassifying patients into their risk categories due to various reasons such as patient characteristics, types of medical conditions, healthcare-related factors, the severity of outcome, and the difference in the association of coefficients between the development and validation cohorts (239,242). Despite the convincing evidence pointing to the poor calibration of SAPS 3 in COVID-19 patients, which undermines its clinical utility, it is being utilized in many countries to monitor and evaluate their prognosis.

Because the aforementioned factors hinder the performance of SAPS 3, the literature strongly recommends its validation and recalibration before administering it to any patient population (71,72). As one research group had already evaluated the performance of SAPS 3 in a smaller cohort of Austrian COVID-19 patients (72); we reassessed the performance of their recalibrated equation in our cohort. Compared to both global and regional versions, the Austrian equation accurately predicted in-hospital mortality, as evident by the SMR value ~1. In addition, it demonstrated better calibration, particularly in patients with diabetes, compared to the other two versions. However, this equation overpredicted mortality in high-risk strata despite being a recalibrated version, especially in patients without diabetes. This pattern of miscalibration is opposite to global and regional versions of SAPS 3 from our cohort and a previous study in Austrian COVID-19 patients (72). Specifically, both uncalibrated global and central versions faced significant departures from calibration in lower-risk strata of COVID-19 patients. We contemplate that the recalibration of the Austrian equation for low-risk strata could have introduced miscalibration in high-risk strata of COVID-19 patients, which is apparent in our study. Furthermore, selective calibration achieved by the Austrian version in patients with diabetes is intriguing, given that it is not a part of this score. We speculate that several factors

could be responsible for this phenomenon. First, diabetes increases the risk of severe COVID-19 infection, which could induce more pronounced derangements in physiological parameters than those without diabetes (101,230). Second, comorbidities and risk factors included in this tool occur more frequently in people with diabetes (173). These reasons may also explain the better performance of uncalibrated equations of SAPS 3 in patients with diabetes. Regardless, even recalibration of SAPS 3 could not fully address the issue of accurate risk stratification of COVID-19 patients. Hence, we recommend that clinicians need to adopt a cautious approach while deciding on clinical management for COVID-19 patients solely based on this tool.

We hypothesized that the SAPS 3 would perform better in patients with diabetes than those without diabetes due to their dysfunctional immune system, advanced age, and a higher prevalence of various chronic medical conditions and metabolic abnormalities (104,173,230). Contrary to our hypothesis, the SAPS 3 discrimination differed marginally (2%) between diabetes and non-diabetes patients, which could be due to an intrinsic limitation of this tool. For instance, the SAPS 3 is a comprehensive prognostication tool that does not capture disease-specific risk factors and biomarkers. In addition, many patient-related and clinical parameters of SAPS 3 such as types of surgical procedures and interventions might be irrelevant for diabetes, acute medical conditions, and severe infections (70). Hence, based on these reasons and our findings, we believe that SAPS 3 may not be a reliable risk stratification and prognostication tool for COVID-19 patients.

This study is not without limitations. Diabetes was defined as per ICD-10 codes; therefore, we refrained from classifying diabetes into subtypes because of possible miscoding. Next, the outcome of in-hospital mortality was defined as death due to any underlying cause. According to this definition, deaths unrelated to COVID-19 could have been included in the outcome. However, the chances of including non-COVID-19 related deaths were low in our study because the GÖG database only collects data on COVID-19. Last, our findings regarding the predictive performance of SAPS 3 in COVID-19 patients may not be transferrable to other cohorts because of differences in the characteristics of patients, distribution and severity of SAPS 3 parameters, and healthcare resources and infrastructure.

In Austrian patients admitted to ICUs for COVID-19, the SAPS 3 demonstrated unsatisfactory discrimination and forecasting power. The predictive power of SAPS 3 was similar in patients with and without diabetes. Both global and regional versions of SAPS 3 encountered a significant departure from calibration, especially in COVID-19 patients without diabetes. Thus, we recommend more research exploring the reasons behind the unsatisfactory predictive performance of SAPS 3 COVID-19 patients. Although the Austrian version of SAPS

3 was reasonably calibrated in COVID-19 patients with diabetes, it demonstrated poor discrimination and accuracy, indicating that even recalibration of this tool could not guarantee its satisfactory performance. Therefore, we recommend periodic performance evaluation and recalibration of SAPS 3 to adopt changes in the characteristics of the SARS-CoV-2 virus and treatment protocols during the COVID-19 pandemic. Moreover, all versions of SAPS 3 showed superior predictive ability in patients with diabetes than those without it. Therefore, we recommend more studies to identify the reasons behind this occurrence. It would also be interesting to compare the predictive performance of SAPS 3 with COVID-19-specific tools in severely ill patients.

5.5. Humoral immune response to COVID-19 vaccination in people with diabetes mellitus

In this COVAC-DM cohort study, we investigated the impact of T1DM, T2DM, and glycaemic control on humoral immune response, measured as SARS-CoV-2 S RBD antibodies, following the first and second vaccination for COVID-19. We also evaluated and compared the frequency of COVID-19 vaccination-related side effects between types of diabetes. In addition, we compared the post-vaccination humoral immune response of people with diabetes against healthy controls. Our study showed that the humoral immune response elicited by COVID-19 vaccination in people with T1DM and T2DM is similar to healthy controls. Regarding the impact of glycaemic control on immune response, the unadjusted analysis showed higher levels of SARS-CoV-2 S RBD antibodies in people with T1DM having well-controlled HbA1c (≤58 mmol/mol) as compared to those with T1DM but insufficiently controlled HbA1c (>58 mmol/mol) as well as both well and insufficiently controlled T2DM. However, this difference in antibody levels between groups became insignificant after adjusting for age and sex and correcting for the false positive discovery rate. Moreover, the correlation analyses of covariates with SARS-CoV-2 S RBD antibodies revealed that age and eGFR significantly predicted humoral immune response in people with diabetes. However, HbA1c was not significantly correlated with antibodies.

Humoral antibody response elicited by people with T1DM and T2DM was similar to healthy controls in our study, which is not in accordance with a previous CAVEAT study. The CAVEAT study demonstrated that people with T2DM and poor glycaemic control (HbA1c >53 mmol/mol [7.0%]) produced a weaker humoral immune response to COVID-19 vaccination compared to people with T2DM having good glycaemic control (HbA1c <53 mmol/mol) and

those without diabetes (159). However, in this study, the cut-off value used to define glycaemic control was lower (53 mmol/mol [7%]) than the cut-off value used in our study (58 mmol/mol [7.5%]). Despite this difference, the mean HbA1c value in people with poorly controlled type 2 diabetes reported by the CAVEAT study (65 ± 2 mmol/mol) is similar to the mean HbA1c value of 68 ± 9 in our cohort. However, to yield more comparable results with the CAVEAT study, we performed a sensitivity analysis by using the cut-off value of HbA1c (53 mmol/mol [7%]) specified in the CAVEAT study to define glycaemic control. Our newer results remained consistent with our previous results, where we used the HbA1c cut-off value of 58 mmol/mol (7.5%), suggesting that the difference in the results between the CAVEAT and our study was not due to the cut-off values used to define glycaemic control. We contemplate that our results differ from the CAVEAT study because of different immunoassays to measure antibody levels. The CAVEAT study used the GenScript SARS-CoV-2 surrogate virus neutralization test, while we adopted Roche Elecsys anti-SARS CoV-2 S assay method. Despite this potential reason for yielding different results, a previous study compared 12 immunoassays for diagnosing COVID-19. According to this study, all 12 assays exhibited a satisfactory positive percent agreement of 84% for diagnosing COVID-19. Nevertheless, positivity rates and seroconversion of SARS-CoV-2 antibodies varied between these immunoassays because of the severity of COVID-19 infection, antigen target, and assay kits (244). While another study by Rubio-Acero directly compared the GenScript SARS-CoV-2 method with the Roche Elecsys anti-SARS CoV-2 S method and reported a satisfactory correlation between these assays having a high positive percent agreement of 90% (245).

As mentioned above, people with T1DM with HbA1c <58 mmol/mol (7.5%) had higher anti-SARS-CoV-2 S antibodies as compared to other groups in our study. However, after adjusting for age, this difference no longer remained significant. This finding indicates that observed higher antibody levels in people with T1DM are not per se due to diabetes, but because they were significantly younger compared to people with T2DM and healthy controls. This finding is further confirmed in our correlation analysis of age with antibody levels, which demonstrated that antibody levels progressively lowered with increasing age. Both findings strongly suggest that age significantly predicts the immune response following COVID-19 vaccination. This finding is in line with a meta-analysis comprising five studies, which compared the efficacy of the COVID-19 vaccine in terms of neutralizing antibody levels among different age groups. This meta-analysis reported that the geometric mean titer values were significantly higher in younger adults in comparison with older adults (SMD: 1.40, $p < 0.01$) (246). Likewise, a recent review summarized various factors influencing humoral and cellular immune response to vaccination. In this review, old age was recognized as a significant

influencing factor for vaccination. In addition, older people produced diminished humoral and cellular immune responses to hepatitis A and B, diphtheria, tetanus, trivalent influenza, tick-borne encephalitis, and pneumococcal polysaccharide vaccines. Furthermore, antibody levels declined more rapidly in older adults than in young adults (247).

Besides age, renal function measured as eGFR was positively correlated with anti-SARS-CoV-2 S antibodies in our cohort. This finding concurs with a previous study on hepatitis B vaccination, which demonstrated that seroconversion rates drop from 95% in healthy subjects to 40–50% in people with stage III to IV CKD (248). A large review article further confirms the significant impact of renal function or CKD on immune response against vaccination. According to this review, people with chronic renal failure and hemodialysis who also had concurrent diabetes elicited a weak immune response to diphtheria, hepatitis B, and tetanus vaccination with a rapid decline in antibodies (247). This consistent body of evidence on various vaccines, including our data on COVID-19, suggests that renal function should be assessed and considered in vaccination planning and administration.

The COVAC-DM study has a few limitations that must be considered while interpreting its findings. We initially planned to enroll a minimum of 40 participants in each group of well- and insufficiently controlled T1DM and T2DM. However, the desired number could not be achieved for people with insufficiently controlled T1DM despite our utmost recruitment efforts. Another limitation is related to the type of immune response we measured in our study. Specifically, we only measured the humoral immune response against the S-protein RBD in this study. Unfortunately, we could not measure the cellular immune response following the COVID-19 vaccination due to uncontrollable supply chain issues in obtaining density gradient collection and falcon tubes for cell isolation at the time of sample collection. Despite not measuring the cellular immune response, a previous study supports our choice of measuring the anti-SARS-CoV-2 S RBD antibodies by demonstrating that neutralizing antibody levels strongly predict immune protection from symptomatic SARS-CoV-2 infection (249). Moreover, we used Roche Elecsys anti-SARS-CoV-2 S immunoassay to measure antibodies in our study and several studies have confirmed that this immunoassay correlates well with neutralizing assays of anti-SARS-CoV-2 (245,250–252). Despite this evidence, there is a possibility that our results may vary when other immunoassays are used to measure antibody levels. Another limitation of this study is related to the availability of antibody data after 3rd COVID-19 vaccination and long-term follow-up data. Given that this study is still running, we plan to collect data related to humoral and cellular immunity for future visits. These data will allow us to elucidate long-term changes in immunity in response to COVID-19 vaccination. As vaccination type may significantly influence immune response, 96% of our study participants received an

mRNA-based vaccine. This distribution of vaccination administration is aligned with the national vaccination strategy of Austria and Germany for people with diabetes. Moreover, Austria plans to administer only mRNA-based vaccines to its population. We therefore could not compare the immune response between types of vaccines. Further to the type of vaccination, in our study, 97% of healthy controls received the Moderna vaccine, whereas 86% of people with diabetes received the BioNTech-Pfizer vaccine, with only 9% receiving the Moderna vaccine. Although both vaccines are mRNA-based, some evidence suggests that the Moderna vaccine produces higher antibodies than Pfizer. Despite this bias in our study, the anti-SARS-CoV2-S antibody levels in healthy controls were not significantly higher than in those with diabetes (253,254).

In conclusion, the COVID-19 vaccination elicited a similar humoral immune response between T1DM, T2DM, and healthy controls after accounting for the impact of age and false positive discovery rate. Likewise, glycaemic control measured by HbA1c had no significant impact on the humoral immune response following COVID vaccination. While age and renal function significantly predicted immune response in our study. We recommend studies investigate both humoral and cellular immune responses to COVID-19 vaccination and long-term changes in antibody levels to provide evidence on determining the exact re-vaccination schedules depending on the presence of specific risk factors.

5.6. Impact of COVID-19 vaccination on glycemia, insulin dosing pattern, and carbohydrate intake in people with diabetes mellitus

In this COVAC-DM substudy, we investigated the short-term effects of the first COVID-19 vaccination (2 days before and 3 days after the vaccination) on glycemia in people with T1DM and T2DM. We also assessed whether the presence and severity of typical side effects following COVID-19 vaccination altered glycemia and influenced bolus insulin dosing and carbohydrate intake patterns in people with diabetes. We found that the vaccination per se did not alter glycemia, carbohydrate intake, and bolus insulin dosing in people with T1DM and T2DM. Nevertheless, people with T1DM spent less time in the near-physiological glucose range (70-180 mg/dL) on days with side effects, while the time spent in hyperglycemia (>180 mg/dL) increased on those days.

Only a limited number of studies have assessed glycaemic excursions around the COVID-19 vaccination period in people with T1DM and T2DM. A similar study in people with

T1DM reported a significant decrease in the mean level of interstitial glucose 7-days after the COVID-19 vaccination as compared to the pre-vaccination period (52% ±2% vs. 55% ±2%, p = 0.030). In addition, a reduction in TIR was observed in 58% of individuals within one week after the vaccination (255). Likewise, a previous case series reported that three people experienced acute hyperglycaemic crisis 3–5 weeks after receiving the first adenovirus-vector COVID-19 vaccine (168). Another case study also described an acute deterioration in glycaemia in a patient with T2DM one week after receiving the second dose of the Pfizer-BioNTech COVID-19 vaccine (256). Similarly, a study reported three cases (1 female and 2 males) of well-controlled diabetes who presented with hyperglycaemia after receiving the first dose of the Covishield™ vaccine. In the first case, the hyperglycaemia persisted for 30 days following the vaccination and normalized after increasing the metformin dosage. The second and third cases developed hyperglycaemia for 3 and 15 days, respectively; however, they did not require any adjustment in the medication (169). However, none of these case studies explicitly investigated the potential influence of COVID-19 vaccination-related side effects on glycemia. We speculate that inflammatory and cytokine responses and subsequent insulin resistance increase on the days with side effects, which may deteriorate glycaemic control (257). However, people with T1DM did not adjust their insulin dosage accordingly on days with side effects, which led to a reduced TIR and increased TAR, respectively.

Our finding concerning the influence of COVID-19 vaccination-related side effects on glycaemia has important clinical implications. As such, people with T1DM who experience severe or cumulative side effects after receiving the first dose of the COVID-19 vaccine can expect a higher demand for exogenous insulin delivered via bolus insulin dose corrections or an increased basal rate/basal insulin dose. Given that the side effects lasted only a few days, the adjustment in the bolus insulin dose seems clinically more reasonable than the basal insulin dose (258). However, based on our findings regarding the insulin dosing adjustment, it seems that there is no need to increase insulin doses after the COVID-19 vaccination in people with T2DM for a long period, but only on those days when side effects were present. Intriguingly, in people with T2DM, the TIR, TAR, and TBR remained unaltered after the vaccination, and the side effects had no impact on glycemia. Considering this, people with T2DM might continue to perform regular therapy management, facing no or little risk of a deterioration in glycemia, even on days of experiencing side effects related to the vaccine. Nevertheless, we recommend more studies to validate our findings in larger patient cohorts.

Our study encounters several limitations. First, our sample size was relatively small (n=74). Therefore, we suggest caution in extrapolating our findings to the entire population of diabetes within and outside Austria. Notwithstanding, our data provide the first evidence on

the impact of COVID-19 vaccination on glycaemic changes in people with diabetes, which could serve as a guide for health care professionals in the field of diabetology while counseling their patients regarding glucose management around vaccination. Second, study participants received different types of COVID-19 vaccines in our study as a part of the national vaccination strategy of Austria and Germany. However, because of the small sample size, we could not stratify our analysis by vaccination types. More than 90% of the study participants received an mRNA vaccine; therefore, our results can be considered representative of this vaccine type. Third, we investigated the glycemic excursions around the first vaccination only due to the unavailability of CGM data around the second vaccination. Our results could have been different for the second vaccination because research shows that typical side effects occur more frequently after the second vaccination in those who received mRNA vaccines (161,162). Last, except one, all participants received continuous subcutaneous insulin or MDI in this sub-study. Therefore, our findings may not apply to people with T2DM being treated with oral glucose-lowering medicines or diet only.

In conclusion, our analysis demonstrated that COVID-19 vaccination as such did not significantly alter glycaemic control in people with T1DM and T2DM. Notably, deterioration in glycaemia was evident in people with T1DM on the days of side effects. Specifically, the glycaemic deteriorations were most pronounced if fever was present together with other side effects, including injection site reactions, headache, body pain, or fatigue. In people with T2DM, side effects had no significant impact on glycaemic control. During the vaccination period, neither any adjustments were required in insulin dosage, nor any changes were noted in carbohydrate intake in people with both T1DM and T2DM. Based on our findings, we recommend large prospective studies to investigate glycaemic excursions around the first and second COVID-19 vaccination and target people treated only with oral antidiabetic medication. In addition, more studies are required to investigate the impact of COVID-19 vaccination-related side effects on glycaemic control in people with T1DM.

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7. APPENDIX

This section contains published versions of following articles that have been included in this cumulative dissertation:

1. Aziz F, Aberer F, Moser O, Sourij C, von Lewinski D, Kaser S, Reichardt B, Sourij H. Impact of comorbidities on mortality in hospitalized influenza patients with diabetes - Analysis of the Austrian Health Insurance. *Diabetes Research and Clinical Practice.* 2021;174:108758. doi: 10.1016/j.diabres.2021.108758.

2. Aziz F, Aberer F, Bräuer A, Ciardi C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. COVID-19 In-Hospital Mortality in People with Diabetes Is Driven by Comorbidities and Age-Propensity Score-Matched Analysis of Austrian National Public Health Institute Data. *Viruses*. 2021;30;13(12):2401. doi: 10.3390/v13122401.

3. Aziz F, Reisinger AC, Aberer F, Sourij C, Sourij H et al., on behalf of the COVID-19 in Diabetes in Austria Study Group. Simplified Acute Physiology Score 3 Performance in Austrian COVID-19 Patients Admitted to Intensive Care Units with and without Diabetes. *Viruses*. 2022;14(4):777. doi: 10.3390/v14040777.

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7.1. Appendix 1

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Impact of comorbidities on mortality in hospitalized influenza patients with diabetes – Analysis of the Austrian Health Insurance

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ABSTRACT

Aims: To assess the impact of characteristics and comorbidities on the hospitalization rate and 30- and 90-days all-cause mortality after hospitalization for influenza-related illness (IRI) in individuals with diabetes.

Methods: Data of 507,184 individuals with diabetes enrolled in the national Austrian Health Insurance database during 2013–2017 were analyzed. Hospitalization for IRI was defined as per *International Classification of Disease* 10 codes (J09, J10, J11). All-cause mortality was calculated for 30- and 90-days post-hospitalization.

Results: Of the total diabetes population, 1994 (0.4%) were hospitalized for IRI during 2013–2017. The rate of comorbidities was higher in individiuals who were hospitalized due to IRI as compared with the general diabetes population. Overall 30-days cumulative mortality following hospitalization for IRI was 7.9% and 90-days mortality was 10.3%. The risk (adjusted Hazard Ratio, 95% Confidence Interval) of IRI related 90-days mortality increased with age (50–59: 3.00, 0.65–13.94; 60–69: 4.16, 0.99–17.55; 70–79: 4.79, 1.16–19.76; 80+: 7.15, 1.74–29.46), heart failure (1.97, 1.31–2.98), renal disease (1.50, 1.05–2.14), and Charlson comorbidity index (1.14, 1.08–1.19).

Conclusions: Older age, heart failure, renal disease, and Charlson comorbidity index were significant predictors of mortality following hospitalization for IRI in individuals with diabetes. These findings could help in improving the clinical management and performance of surveillance and health systems concerning IRI in Austria.

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1. Introduction

Individuals with diabetes face an elevated overall risk of infections that can be attributed to impaired phagocytosis by neutrophils, macrophages, and monocytes, impaired neutrophil chemotaxis, and bactericidal activity as well as impaired innate cell-mediated immunity [1,2]. Chronic hyperglycemia *per se* has been shown to promote immune dysfunction [1,2], micro- and macro-angiopathies [3], neuropathic disorders [4], inhibits antibacterial activity [5], and impairs gastrointestinal and urinary motility [6]. Also, it increases the risk for diabetes related hospitalizations [7] and the necessity for medical interventions, which altogether make them more susceptible to nosocomial and community-acquired infections [8].

During the last years, various countries worldwide experienced endemic waves of seasonal flu infection episodes with remarkable rates of related deaths [9]. The most common pathogenic disease trigger is influenza (A and B) virus, which represents a significant healthcare challenge as every year approximately 5—15% of the global population is infected and data from the *World Health Organisation* (WHO) suggest that an estimated half a million of people per year die from respiratory diseases in association with influenza virus infections [10,11]. Important to note, older age (≥65 years), as well as geographical and socioeconomic diversity, have been announced as the most prominent predictors of influenza-related mortality [12].

With regard to medical conditions, an interaction between chronic diseases and risk of influenza virus infection has been frequently described [13], and in particular individuals with diabetes have a three- and four-fold increased risk for hospitalization and admission to intensive medical care units respectively due to influenza virus-induced complications [14]. In a cohort from Germany, almost 20% of the fatal influenza cases were attributed to diabetes, with pre-existing diabetes doubling the risk for a fatal outcome [15]. Regardless of the type and patient constitution, individuals with diabetes are referred to as the key risk group for virus infections like the seasonal flu or pandemic outbreaks like SARS-CoV-2. Considering that individuals with diabetes represent a very heterogeneous group of patients including various types, typically compromised by potential comorbidities of different extent, it remains unanswered what specific characteristics in people with diabetes increase the risk of fatal influenza virus infection.

This nationwide retrospective analysis aimed to identify diabetes-related characteristics and comorbidities associated with hospitalization rate as well as 30- and 90-days mortality after hospitalization following the diagnosis of influenza-related illness (IRI) using the Austrian health insurance (AHI) database.

2. Methods

2.1. Study design and setting

The '*Strengthening the Reporting of Observational studies in Epidemiology (STROBE)*' checklist was used for reporting this study [16]. It was a retrospective cohort study of individuals diagnosed with diabetes who were enrolled in the AHI database. The AHI provides health care to approximately 99% of the Austrian population and compiles patient data from 12 regional health insurances, which is provided by participating healthcare facilities across Austria. The database for individuals with diabetes only contains pseudo-anonymized information on demographic characteristics, primary and secondary medical diagnosis provided as *International Classification of Disease* (ICD) codes along with discharge dates from hospitals, prescribed medications coded as *Anatomical Therapeutic Classification* (ATC) system introduced by the *World Health Organization*, along with their dosage, volume, start dates, and end dates, medical procedures stored as MEL codes, and all-cause mortality along with the date of death.

2.2. Ethical considerations

This study was approved by the ethics committee of the Medical University of Graz (approval number: 32–370 ex 19/20) and was conducted in full conformity with the 1964 declaration of Helsinki as well as in accordance with the guidelines laid down by the International Conference on Harmonization for Good Clinical Practice (ICH GCP E6 guidelines). Since this is an analysis of pseudonymised health insurance data, no informed consent was obtained from the patients.

2.3. Data extraction and study variables

This study used the AHI data of 507,184 individuals with diabetes (type 1 diabetes and type 2 diabetes) who were hospitalized during the period of January 1st, 2013 and December 31st, 2017. The diagnosis of diabetes was confirmed using both the ICD-10 and ATC codes of glucose-lowering medicines (Supplementary Table 1). Information regarding both primary and secondary IRI hospitalizations was extracted from the data using the ICD-10 codes of J09, J10, and J11. As confirmatory tests for influenza were not performed in all patients, misclassifications of codes were possible, and these codes may also have included influenza-like illnesses. The date of hospitalization was not recorded in the data; therefore, the date of discharge was used to estimate the time-to-mortality instead. All-cause mortality was defined and calculated as the mortality occurring within 30-days and 90-days of discharge from the hospital respectively. The death dates were available in the database for all people who died either in the hospital or after discharge (no matter where they died). Therefore, patients who died in the hospital (their discharge date usually equals the death date) were also included in the analysis. As multiple episodes of IRI and subsequent dates of discharge from hospital were identified for each patient, the first date of discharge was considered to identify the first hospitalization related to influenza-related illness. While subsequent discharge dates reported within a month following the first or preceding episodes were excluded to capture the correct number of influenza episodes per year for each patient. The age-adjusted *Charlson Comorbidity Index* (ACCI), a validated weighted summary score, was generated using the ICD-10 codes of various comorbidities with age added as an

additional risk factor. These comorbidities included myocardial infarction, congestive heart failure (CHF), peripheral vascular disease, stroke, dementia, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, peptic ulcer disease, liver disease, diabetes, hemiplegia, renal disease, cancer, and acquired immune deficiency syndrome (AIDS) [17]. The ACCI score was further categorized into 0–3, 4–6, 7–9, and 10+ scores. Patients were classified into type 1 diabetes and type 2 diabetes using the ICD-10 codes. The age of individuals was calculated from the date of birth until the date of discharge from the hospital after the first episode of IRI. The information regarding sex of participants was recorded in the AHI database.

2.4. Statistical analysis

Statistical analysis was performed in Stata version 16.1 (StataCorp LLC). Quantitative variables were summarized as mean ± standard deviations (±SD) and qualitative variables as frequencies and percentages (%). Simple and multiple logistic regression analyses were performed to assess unadjusted and adjusted associations of characteristics, comorbidities, and the ACCI with IRI hospitalizations. Three multiple logistic regression models were performed: model I included characteristics, type of diabetes, and individual comorbidities as covariates; model II included sex, type of diabetes, and the ACCI score as covariates; and model III included sex, type of diabetes, and the categorical ACCI as covariates. The results of logistic regressions were reported as odds ratios (OR) and adjusted odds ratios (aOR) with corresponding 95% confidence intervals (CI) and p-values. Distribution of hospitalized cases of IRI by month was shown in a line graph. Cumulative 30- and 90-days mortality after IRI, overall and stratified by characteristics, comorbidities, and the ACCI, were reported as % with corresponding 95%CI. Log-rank tests were performed to compare mortality rates. Simple and multiple multilevel parametric survival regression analyses were performed to assess the unadjusted and adjusted associations of characteristics, individual comorbidities (model I), and the ACCI score (model II) with 90-days IRI mortality using the number of episodes of IRI and types of insurances as random effects in the model. In addition, interaction effects between characteristics and comorbidities were assessed by adding their interaction terms in the model I. The results of the multilevel survival regression were reported as hazard ratios (HR) and adjusted hazard ratios (aHR) with corresponding 95%CI and p-values.

3. Results

3.1. Patients hospitalized for influenza-related illness versus non-influenza cohort

A total of 1,994 individuals and 2,014 cases (including readmissions) with established diabetes were hospitalized due to IRI at various Austrian hospitals between 2013 and 2017. Of the total individuals, 50.4% were coded as J10, 29.4% as J11, and 21.2% as J09. The seasonal distribution of IRI cases showed the highest number of hospitalizations from November to February (Supplementary Fig 1). Compared to the cohort (505,190) of non-influenza related hospitalizations, the influenza-affected cohort was older (72.6 ± 14.3 vs. 68.9 ± 15.1 years, p <0.001) and had more frequent comorbidities such as cancer, COPD, CHF, dementia, liver disease, and renal disease (Table 1). The adjusted odds ratio for influenza-related hospitalizations was the highest when COPD was present (aOR: 2.85, 95%CI: 2.56–3.18) followed by renal disease (aOR: 2.12, 95%CI: 1.89–2.37). In particular, the accumulation of comorbidities expressed by the ACCI was associated with increased adjusted odds (aOR: 1.25, 95%CI: 1.24–1.27) for influenza-related hospitalizations. The majority of individuals were coded as type 2 diabetes (96%) within the whole cohort. Individuals with type 1 diabetes demonstrated 33% higher adjusted odds (95%CI: 1.11–1.59) for being hospitalized due to IRI as compared to those with type 2 diabetes (Table 1).

3.2. Mortality following hospitalization for an influenza-related illness

The median follow-up time was 714 days. The overall 30-days mortality following hospitalization due to IRI was 7.9% (n = 158, 95%CI: 6.8–9.1%), while 90-days mortality was 10.3% (n = 205, 95%CI: 9.0–11.7%). Both 30-days and 90-days mortality rates significantly increased with age (p = 0.003, p < 0.001). In individuals with CHF (p = 0.013, p < 0.001), dementia (p = 0.026, p = 0.008), and renal disease (p = 0.059, p = 0.004), 30-days and 90-days mortality rates were also significantly higher in those with the co-morbidity than in those without. A numerical, albeit not statistically significant lower mortality rate was observed in individuals with type 1 diabetes when compared against those with type 2 diabetes (Table 2).

Within the multiple survival regression analysis (Table 3), increasing age was significantly associated with mortality risk, as were the presence of CHF (aHR: 1.97, 95%CI: 1.31–2.98), and renal disease (aHR: 1.50, 95%CI: 1.05–2.14). The comorbidity COPD was not associated with increased mortality risk. Likewise, the type of diabetes (either type 1 or type 2) had no impact on 90-days mortality. The ACCI was the strongest predictor of mortality with an average increase of 14% in mortality risk with each score point increase in the ACCI.

4. Discussion

This is the first nationwide analysis of characteristics of individuals with diabetes who were admitted to the hospital due to IRI. Individuals hospitalized due to IRI were older, more likely to be male, and had a higher number of comorbidities as compared to those not hospitalized due to IRI. Our findings regarding the impact of age on the hospitalization rate of IRI are in line with previous population-based research, which showed higher admission rates among the elderly [18,19].

Although in our analysis individuals admitted to the hospital due to IRI were on average only ~4 years older than those admitted to the hospital due to other causes, older age was also a strong predictor for the 30- and 90-days all-cause mortality following IRI. For instance, individuals with diabetes aged above 80 years compared to those aged below 50 years,

Table 1 – Comparison of characteristics between individuals hospitalized for influenza-related illness and other causes (N = 507,184).

Variables	With Influenza (N = 1994)	Without Influenza (N = 505,190)	Simple Regression		Multiple Regression	
	Freq (%)	Freq (%)	OR (95%CI)	P-Value	aOR (95%CI)	P-Value
			Model I			
Age categories						
<50 years	89 (4.5)	47,994 (9.5)	Reference		Reference	
50–59 years	154 (7.7)	67,695 (13.4)	1.23 (0.95–1.59)	0.123	1.14 (0.88–1.49)	0.318
60–69 years	293 (14.7)	115,032 (22.8)	1.37 (1.08–1.74)	0.009	1.14 (0.90–1.45)	0.286
70–79 years	654 (14.7)	143,777 (28.5)	2.45 (1.96–3.06)	<0.001	1.74 (1.39–2.19)	<0.001
80+ years	804 (32.8)	130,844 (25.9)	3.31 (2.66–4.12)	<0.001	2.01 (1.60–2.52)	<0.001
Sex						
Male	1085 (54.4)	255,062 (51.2)	Reference		Reference	
Female	909 (45.6)	243,128 (48.8)	0.89 (0.81–0.98)	0.014	0.85 (0.78–0.93)	0.001
Type of Diabetes						
Type 2 diabetes	1858 (93.2)	485,683 (96.1)	Reference		Reference	
Type 1 diabetes	136 (6.8)	19,507 (3.9)	1.74 (1.45–2.09)	<0.001	1.33(1.11–1.59)	0.002
Comorbidities						
Cancer (+/−)	323 (16.2)	45,839 (9.1)	2.04 (1.80–1.30)	<0.001	1.35 (1.20–1.53)	<0.001
COPD (+/−)	569 (28.5)	41,694 (8.3)	4.56 (4.13–5.04)	<0.001	2.85 (2.56–3.18)	<0.001
CHF (+/−)	471 (23.6)	44,346 (8.8)	3.25 (2.93–3.62)	<0.001	1.26 (1.12–1.43)	<0.001
Dementia (+/−)	253 (12.7)	22,741 (4.5)	3.14 (2.75–3.60)	<0.001	1.61 (1.40–1.86)	<0.001
Liver disease (+/−)	242 (12.1)	30,387 (6.0)	2.23 (1.94–2.56)	<0.001	1.37 (0.95–1.97)	<0.094
Renal disease (+/−)	648 (32.5)	54,398 (10.8)	4.06 (3.68–4.47)	<0.001	2.12 (1.89–2.37)	<0.001
			Model II			
ACCI score, Mean ± SD	5.2 ± 2.7	4.0 ± 2.9	1.25 (1.24–1.27)	<0.001	1.25 (1.24–1.27)	<0.001
			Model III			
ACCI score						
0–3	527 (24.4)	255,035 (50.5)	Reference		Reference	
4–6	859 (43.1)	157,045 (31.1)	4.52 (3.91–5.22)	<0.001	4.44 (3.84–5.12)	<0.001
7–9	485 (24.3)	65,335 (12.9)	9.73 (8.41–11.25)	<0.001	9.46 (8.17–10.95)	<0.001
10+	123 (6.2)	27,775 (5.5)	10.45 (8.82–12.37)	<0.001	10.00 (8.43–11.85)	<0.001

OR: Odds ratio, aOR: Adjusted Odds Ratio, CI: Confidence Interval, SD: Standard Deviation, COPD: Chronic Obstructive Pulmonary Disease, CHF: Congestive Heart Failure, ACCI: Age-adjusted Charlson comorbidity Index.

Continuous variables are presented as mean ± SD and categorical variables are presented as n (%).

Model I: Included age, sex, diabetes, and comorbidities. Adjusted estimates of age, sex, diabetes, and comorbidities are reported only for this model.

Model II: Included ACCI (continuous variable) sex, and diabetes. Adjusted estimates are reported only for ACCI.

Model III: Included ACCI (categorical variable), sex, and diabetes. Adjusted estimates are reported only for ACCI.

Table 2 – Cumulative mortality after hospitalization for influenza-related illness (N = 1994).

Variables	30-days mortality (N = 158)		90-days mortality (N = 205)	
	% (95%CI)	P-Value	% (95%CI)	P-Value
Overall	7.9 (6.8–9.1)	NA	10.3 (9.0–11.7)	NA
Age				
<50 years	1.7 (0.4–6.4)	0.003	1.7 (0.4–6.4)	<0.001
50–59 years	5.2 (2.8–9.8)		5.2 (2.8–9.8)	
60–69 years	6.7 (4.6–9.7)		8.3 (5.9–11.6)	
70–79 years	7.5 (5.6–9.8)		9.7 (7.6–12.2)	
80+ years	10.7 (8.5–13.3)		14.9 (12.4–17.8)	
Sex				
Male	8.7 (7.1–10.5)	0.219	10.9 (9.2–12.9)	0.385
Female	6.9 (5.5–8.8)		9.6 (7.8–11.7)	
Type of Diabetes				
Type 1 diabetes	3.7 (1.5–8.6)	0.059	5.9 (3.0–11.4)	0.081
Type 2 diabetes	8.2 (7.0–9.5)		10.6 (9.3–12.1)	
Type of Influenza				
J09	8.1 (5.8–11.1)	0.166	9.2 (6.8–12.4)	0.169
J10	8.8 (7.2–10.7)		11.5 (9.7–13.7)	
J11	6.2 (4.5–8.5)		8.8 (6.8–11.5)	
Comorbidities				
Cancer				
No	7.7 (6.5–9.1)	0.560	9.7 (8.4–11.2)	0.054
Yes	8.7 (6.1–12.3)		13.3 (10.1–17.5)	
COPD				
No	7.9 (6.6–9.4)	0.866	9.9 (8.5–11.6)	0.467
Yes	7.9 (6.0–10.5)		11.1 (8.8–13.9)	
CHF				
No	7.1 (5.9–8.5)	0.013	8.9 (7.6–10.5)	<0.001
Yes	10.4 (8.0–13.5)		14.7 (11.8–18.2)	
Dementia				
No	7.3 (6.2–8.7)	0.026	9.6 (8.3–11.1)	0.008
Yes	11.5 (8.1–16.1)		15.0 (11.2–20.1)	
Renal disease				
No	7.1 (5.8– 8.6)	0.059	8.9 (7.5–10.6)	0.004
Yes	9.6 (7.5–12.1)		13.1 (10.7–16.0)	
Liver disease				
No	7.9 (6.7–9.2)	0.967	10.5 (9.1–12.0)	0.528
Yes	7.8 (5.1–12.0)		9.1 (6.1–13.5)	
ACCI				
0–3	3.4 (2.2–5.4)	<0.001	3.6 (2.3–5.6)	<0.001
4–6	9.4 (7.7–11.6)		11.5 (9.6–13.9)	
7–9	9.5 (7.2–12.5)		13.6 (10.9–17.0)	
10+	9.8 (5.7–16.5)		17.1 (11.5–25.0)	

CI: Confidence Interval, COPD: Chronic Obstructive Pulmonary Disease, CHF: Congestive Heart Failure, ACCI: Age-adjusted Charlson Comorbidity Index, T1DM: Type 1 Diabetes, T2DM: Type 2 Diabetes,
P-values are reported for log-rank test

displayed a cumulative 30-days mortality of 10.7%. vs. 1.7% and 90-days mortality of 14.9% vs. 1.7%. This finding is in line with previous analyses, which showed that older people are at an elevated risk of mortality during IRI [19–22]. Conclusively, a *meta*-analysis assessing risk factors of influenza-induced deaths in the general population showed that older people had higher odds (OR: 2.95, 95%CI: 1.53–5.70) of dying compared to younger counterparts [19].

When assessing the impact of the type of diabetes, individuals with type 1 diabetes had a higher risk for hospital admission compared to those with type 2 diabetes (admission rates 0.67% vs. 0.38%). Although in our dataset individuals with type 1 diabetes were more prone to hospitalization due to IRI, mortality rates and adjusted hazard rates for 90-days mortality were similar for both types of diabetes. A recent Norwegian analysis concluded that hospitalization rates need to be interpreted with caution, as in their analysis, hospitalization for influenza was shown to be more common in individuals with type 2 diabetes than those without diabetes [23]. The AHI does not collect information on diabetes duration or measures of glycemic control (e.g. HbA_{1c}). Hence, we could not assess the impact of these characteristics on hospitalization rates and mortality. As shown in a recent *in vitro* and *in vivo* animal study, fluctuations in blood glucose levels seem

Table 3 – Simple and multiple multilevel survival regression analyses for 90-days all-cause mortality related to influenza-related illness (N = 1994).

Variable	Simple Regression		Multiple Regression	
	HR (95%CI)	P-Value	aHR (95%CI)	P-Value
Model I				
Age categories				
<50 years	Reference		Reference	
50–59 years	3.25 (0.70–15.04)	0.132	3.00 (0.65–13.94)	0.161
60–69 years	5.18 (1.24–21.64)	0.024	4.16 (0.99–17.55)	0.052
70–79 years	6.06 (1.48–24.75)	0.012	4.79 (1.16–19.76)	0.030
80+ years	9.41 (2.32–38.23)	0.002	7.15 (1.74–29.46)	0.006
Sex (Female/Male)	0.85 (0.64–1.13)	0.262	0.79 (0.60–1.05)	0.103
Diabetes (Type 2/Type 1)	1.80 (0.89–3.67)	0.682	1.40 (0.68–2.88)	0.358
Type of influenza				
J10/J09	1.23 (0.85–1.77)	0.273	1.24 (0.86–1.78)	0.248
J11/J09	0.92 (0.59–1.42)	0.696	0.97 (0.64–1.48)	0.890
Cancer (+/–)	1.46 (1.03–2.05)	0.031	1.41 (1.00–1.98)	0.051
COPD (+/–)	1.11 (0.82–1.50)	0.484	1.06 (0.78–1.44)	0.702
CHF (+/–)	1.68 (1.25–2.25)	0.001	1.97 (1.31–2.98)	0.001
Dementia (+/–)	1.61 (1.13–2.29)	0.009	1.24 (0.86–1.80)	0.250
Renal disease (+/–)	1.49 (1.13–1.98)	0.005	1.50 (1.05–2.14)	0.026
Liver disease (+/–)	0.88 (0.57–1.38)	0.584	0.85 (0.54–1.33)	0.468
Model II				
ACCI (continuous)	1.14 (1.08–1.19)	<0.001	1.14 (1.08–1.19)	<0.001

HR: Hazard Ratio, aHR: Adjusted Hazard Ratio, COPD: Chronic Obstructive Pulmonary Disease, CHF: Congestive Heart Failure, ACCI: Age-adjusted Charlson Comorbidity Index, T1DM: Type 1 Diabetes, T2DM: Type 2 Diabetes,
Model I: Included age, sex, diabetes, and comorbidities,
Model II: Included ACCI (continuous variable), sex, and diabetes. Adjusted estimates are reported only for ACCI.

to be associated with severe influenza virus infections [24]. Hence, some of the hospitalizations might have occurred due to unstable glycemia in our study. In line with this observation, it was shown that suboptimal glycemic control increased the risk of infectious diseases and mortality [25–27].

Our data suggest that besides the age, comorbidities and their accumulation are important determinants for hospitalization due to IRI. As such, individuals admitted to the hospital were more likely to have cancer, COPD, CHF, and liver or renal diseases. In line with our analysis, similar comorbidities were documented by a previous study that compared characteristics of individuals with diabetes vs. non-diabetes admitted for influenza. However, in their study, dementia was not included as comorbidity, which was interestingly about three times more common in those being hospitalized for IRI in our data. In contrast to our findings, a study of over 2 million elderly individuals reported that those with dementia and no diabetes surprisingly had a lower frequency of influenza infections and a shorter length of stay in the hospital [28]. In our study, the diagnosis of cancer also increased the likelihood of IRI hospitalization, which is in line with previous research [29]. Within the group of people with diabetes and cancer, it is almost impossible to assess whether cancer *per se* or its pharmacotherapy increases the infection risk and mortality rate following an IRI.

Our analysis showed that the impact of comorbidities like CHF, cancer, and renal disease as well as the ACCI on 90-days mortality post-influenza hospitalization remained significant after adjusting for confounding factors. Renal disease, a common long-term complication of diabetes, was found to increase the risk of mortality following the hospitalization for IRI. Interestingly, this finding is contrary to the results of a *meta*-analysis conducted in individuals without diabetes, in which renal disease was not a significant predictor for mortality. Unsurprisingly, in our study, increasing ACCI was the strongest predictor for mortality following the hospitalization for IRI. This finding is compatible with a recent study that reported a higher ACCI in fatal cases when compared against non-fatal cases in non-diabetic adults [30]. The accumulation of different pre-existing medical conditions potentially increases the risk of multi-organ failure during acute infection which can consequently result in an increased risk of mortality.

The AHI data has various limitations as it relies on the quality of diagnosis coding within the hospital, hence confirmatory influenza test was not available for all subjects. Since these data are not strictly monitored as it is usually done in clinical trials, diseases such as type of diabetes, type and severity of IRI, and other comorbidities might have been missed or inaccurately coded. As the number of people with type 1 diabetes being hospitalized for influenza is small given the lower prevalence as compared to type 2 diabetes, the findings in this patient group need to be interpreted cautiously. Also, this database does not contain the hospital admission date but only the discharge date and we used this date to perform time-to-event analyses. Another limitation is that the database does not capture data on glycemic control, lipids, or other laboratory values, history of previous IRI hospitalizations, status of influenza vaccination, emergency care, and admission to critical care units. Hence, no adjustment for glycemic control, hyperlipidemia, or kidney function could be performed. However, even without the vaccination informa-

tion, which is below 10% for the overall Austrian population, the overall message of the analysis being that age and accumulating comorbidities do impact 90-days mortality following hospitalization for IRI in individuals with diabetes, remains unaltered. Moreover, we believe that our data can be helpful in the future for potential vaccination campaigns, that could focus specifically on the group of people identified in our analysis. The data collected are derived from people with diabetes in Austria and do not include other European countries. As the full availability of the data until the release for research take some time, we were only able to include data up to the end of 2017 at the time of data analyses. However, it is unlikely that the risk factor pattern for fatal outcomes changed over the last years.

This analysis of the AHI database demonstrates a higher rate of comorbidities in individuals hospitalized due to IRI as compared to the general diabetes population in Austria. Of those being hospitalized because of IRI, age and in particular accumulating comorbidities summarized by the ACCI provide a helpful tool to characterize and identify individuals at increased mortality risk. We believe that this analysis can guide clinicians in the identification of groups within people with diabetes who are at the highest risk of hospitalization and developing fatal outcomes following IRI.

Data availability

The dataset generated and analysed in this study is not publicably available but may be obtained from the corresponding author upon a reasonable request.

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Author contributions

H.S. and F.A. designed the interpreted the data, F.A. performed the statistical analysis. F.A., F.Ab., O.M., and C.S. drafted and edited the manuscript. S.K., D.vL., and B.R. helped with interpreting the data and edited the manuscript. H.S. and F.A. are the guarantors of the work and as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of Competing Interests

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2021.108758.

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7.2. Appendix 2

viruses

MDPI

Article

COVID-19 in-Hospital Mortality in People with Diabetes Is Driven by Comorbidities and Age—Propensity Score-Matched Analysis of Austrian National Public Health Institute Data

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Abstract: Background: It is a matter of debate whether diabetes alone or its associated comorbidities are responsible for severe COVID-19 outcomes. This study assessed the impact of diabetes on intensive care unit (ICU) admission and in-hospital mortality in hospitalized COVID-19 patients. Methods: A retrospective analysis was performed on a countrywide cohort of 40,632 COVID-19 patients hospitalized between March 2020 and March 2021. Data were provided by the Austrian data platform. The association of diabetes with outcomes was assessed using unmatched and

propensity-score matched (PSM) logistic regression. Results: 12.2% of patients had diabetes, 14.5% were admitted to the ICU, and 16.2% died in the hospital. Unmatched logistic regression analysis showed a significant association of diabetes (odds ratio [OR]: 1.24, 95% confidence interval [CI]: 1.15–1.34, $p < 0.001$) with in-hospital mortality, whereas PSM analysis showed no significant association of diabetes with in-hospital mortality (OR: 1.08, 95%CI: 0.97–1.19, $p = 0.146$). Diabetes was associated with higher odds of ICU admissions in both unmatched (OR: 1.36, 95%CI: 1.25–1.47, $p < 0.001$) and PSM analysis (OR: 1.15, 95%CI: 1.04–1.28, $p = 0.009$). Conclusions: People with diabetes were more likely to be admitted to ICU compared to those without diabetes. However, advanced age and comorbidities rather than diabetes itself were associated with increased in-hospital mortality in COVID-19 patients.

Keywords: COVID-19; diabetes; intensive care; mortality; SARS-CoV-2

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus that caused a pandemic of coronavirus disease starting in 2019 (COVID-19). As of 27 July 2021, 194.72 million confirmed cases of COVID-19 and 4.17 million deaths have been reported globally with an estimated fatality rate of approximately 2% [1,2].

Earlier research from China, the United States, and Europe has reported a high prevalence of diabetes (17–33%) among people hospitalized for COVID-19 [3–5]. In addition, people with diabetes are more likely to develop severe SARS-CoV-2 infection and complications such as diabetic ketoacidosis and death [2,6,7]. Various mechanisms have been delineated to understand the relationship between diabetes and COVID-19. As such, people with diabetes have a compromised immune system and dysfunctional inflammatory response, which may result in a more complex course of the disease and prolonged recovery. Moreover, SARS-CoV-2 infection may disrupt the regulation of blood glucose in people with diabetes, which provides a thriving environment to the virus, thereby making it challenging to treat this infection [8].

Recent studies have shown that certain risk factors such as old age, sex, and smoking status and the presence of comorbidities such as cardiovascular disease, dementia, liver disease, renal disease, and cancer also significantly increase the risk of developing severe disease and mortality in people with COVID-19 [5,7,9,10]. In particular, type 2 diabetes is a disease of advanced age with a high burden of multimorbidity [11]; it is speculated that the co-existence of these underlying conditions exacerbates the prognosis of COVID-19 in people with diabetes [12]. However, evidence concerning the impact of diabetes on COVID-19 outcomes that is independent of age, sex, and multimorbidity is still emerging [13,14]. Therefore, this study assessed the association of diabetes with the severity of disease leading to intensive care unit (ICU) admission and in-hospital mortality in patients hospitalized for SARS-CoV-2 infection in Austria by adopting a propensity score matching (PSM) method to account for sex, age, and comorbidities.

2. Materials and Methods

2.1. Study Design and Data Source

The 'Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)' checklist was used for reporting this study [15]. A retrospective cohort study was conducted in patients hospitalized for primary and secondary SARS-CoV-2 infection in Austria. These data were collected by the "Data platform COVID-19" provided by the Austrian National Public Health Institute (Gesundheit Österreich GmbH, Stubenring 6, 1010, Vienna, Austria).

This platform captures countrywide epidemiological and clinical data of COVID-19 patients in Austria to improve understanding and provide updated evidence regarding

SARS-CoV-2 infection in the country. The details about the Austrian data platform are available at: https://datenplattform-covid.goeg.at/english, accessed on 3 May 2021.

2.2. Data Extraction and Study Variables

This study includes data of COVID-19 patients with and without diabetes mellitus who were hospitalized between March 2020 and March 2021 in Austria. Of the 51,469 patients recorded in the database, 40,602 patients were included in the final analysis after excluding patients aged below 20 years and with missing and duplicate data (Figure 1). Study variables comprised age deciles at admission, sex, number of hospitalizations for COVID-19, geographic regions, diabetes mellitus, comorbidities, ICU admission, and in-hospital mortality.

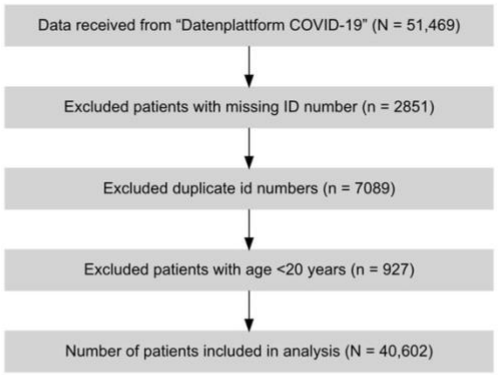

Figure 1. Flow diagram of extraction.

Diabetes mellitus was defined as per the International Classification of Disease (ICD) version 10 codes of E10, E11, E12, E13, and E14. Comorbidities were identified by referring to Charlson and Elixhauser indices and defined as per the ICD-10 codes. The comorbidities included myocardial infarction, cardiac arrhythmias, valvular heart disease, hypertension, congestive heart failure (CHF), peripheral vascular disease, stroke, chronic obstructive pulmonary disease (COPD), pulmonary circulation disorders, dementia, rheumatoid disease, peptic ulcer disease, liver disease, paralysis, other neurological disorders, chronic renal disease, cancer with/without metastasis, Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS), hypothyroidism, coagulopathy, fluid and electrolyte disorders, blood loss anemia, deficiency anemia, alcohol abuse, psychosis, and depression [16,17]. The detailed description of diseases included as comorbidities with their respective ICD-10 codes is provided in Appendix A. In addition to individual comorbidities, the Charlson Comorbidity Index (CCI) was calculated using the 'comorbidity' package to measure the cumulative impact of comorbidities (except diabetes) on outcomes.

2.3. Ethical Considerations

The study was reviewed by the ethics committee of the Medical University of Graz, Graz, Austria (EK 32-355 ex 19/20) and conformed to the 1964 declaration of Helsinki and guidelines of the International Conference on Harmonization for Good Clinical Practice (ICH GCP E6 guidelines). As it was a retrospective analysis of anonymized data, no informed consent was obtained from the patients.

2.4. Statistical Analysis

Data were received in the Microsoft Excel format and analyzed in R version 4.1.0. Qualitative variables were summarized as frequencies with corresponding percentages (%) and compared with diabetes status using Pearson's chi-square or Fischer's exact tests and standardized mean differences (SMD).

The impact of diabetes on both ICU admission and in-hospital mortality was assessed using unmatched and PSM logistic regression. In unmatched logistic regression, the unadjusted association of diabetes with both outcomes was ascertained in the entire cohort (N = 40,602). In PSM analysis, the logistic regression model of diabetes with unbalanced variables having an SMD of ≥0.1 each (Table 1) was fitted to estimate the propensity score. Next, the 'MatchIt' package was used to generate a 1:1 without replacement PSM cohort of diabetes (n = 4971) and non-diabetes (n = 4971) patients by applying the nearest neighbor method. After propensity score matching, the SMD was re-estimated in the PSM cohort to determine whether the balance was achieved across selected variables or not. The results of both unmatched and PSM logistic regression were reported as odds ratios (OR) with corresponding 95% confidence intervals (CI) and *p*-values. Furthermore, the association of diabetes with outcomes was adjusted for variables that remained unbalanced even after performing the PSM. A *p*-value of <0.05 was chosen to determine statistical significance.

Table 1. Characteristics and comorbidities of diabetes and non-diabetes patients hospitalized for COVID-19 patients in unmatched and propensity score matched cohorts.

Variables	Unmatched Cohort					PSM Cohort		
	All	No Diabetes n (%)	Diabetes n (%)	SMD	*p*-Value	No Diabetes n (%)	Diabetes n (%)	SMD
N	40,602	35,631 (87.8)	4971 (12.2)			4971 (50.0)	4971 (50.0)	
Men	21,278 (52.4)	18,419 (51.7)	2859 (57.5)	0.117	<0.001	2839 (57.1)	2859 (57.5)	0.008
Women	19,324 (47.6)	17,212 (48.3)	2112 (42.5)			2132 (42.9)	2112 (42.5)	
Age categories								
20–29	1057 (2.6)	1050 (2.9)	7 (0.1)			5 (0.1)	7 (0.1)	
30–39	1576 (3.9)	1536 (4.3)	40 (0.8)			44 (0.9)	40 (0.8)	
40–49	2479 (6.1)	2306 (6.5)	173 (3.5)			177 (3.6)	173 (3.5)	
50–59	5363 (13.2)	4751 (13.3)	612 (12.3)	0.403	<0.001	643 (12.9)	612 (12.3)	0.028
60–69	6674 (16.4)	5743 (16.1)	931 (18.7)			931 (18.7)	931 (18.7)	
70–79	9664 (23.8)	8133 (22.8)	1531 (30.8)			1490 (30.0)	1531 (30.8)	
80–89	10,532 (25.9)	9144 (25.7)	1388 (27.9)			1387 (27.9)	1388 (27.9)	
90+	3257 (8.0)	2968 (8.3)	289 (5.8)			294 (5.9)	289 (5.8)	
ICU admission	5968 (14.7)	5057 (14.2)	911 (18.3)	0.110	<0.001	812 (16.3)	911 (18.3)	0.053
In-hospital mortality	6569 (16.2)	5632 (15.8)	937 (18.8)	0.080	<0.001	881 (17.7)	937 (18.8)	0.029
Comorbidities								
Myocardial infarction	340 (0.8)	236 (0.7)	104 (2.1)	0.123	<0.001	107 (2.2)	104 (2.1)	0.004
Cardiac arrhythmias	4235 (10.4)	3134 (8.8)	1101 (22.1)	0.376	<0.001	1217 (24.5)	1101 (22.1)	0.055
Valvular heart disease	990 (2.4)	738 (2.1)	252 (5.1)	0.162	<0.001	307 (6.2)	252 (5.1)	0.048
Hypertension	10,538 (26.0)	7209 (20.2)	3329 (67.0)	1.069	<0.001	3002 (60.4)	3329 (67.0)	0.137
CHF	2011 (5.0)	1381 (3.9)	630 (12.7)	0.323	<0.001	602 (12.1)	630 (12.7)	0.017
PVD	1341 (3.3)	855 (2.4)	486 (9.8)	0.312	<0.001	385 (7.7)	486 (9.8)	0.072
Stroke	1212 (3.0)	834 (2.3)	378 (7.6)	0.244	<0.001	378 (7.6)	378 (7.6)	0.002
COPD	2271 (5.6)	1699 (4.8)	572 (11.5)	0.248	<0.001	568 (11.4)	572 (11.5)	0.003
Pulmonary circulation disorders	591 (1.5)	474 (1.3)	117 (2.4)	0.076	<0.001	129 (2.6)	117 (2.4)	0.016
Dementia	2050 (5.0)	1570 (4.4)	480 (9.7)	0.206	<0.001	488 (9.8)	480 (9.7)	0.005
Rheumatoid disease	254 (0.6)	198 (0.6)	56 (1.1)	0.063	<0.001	77 (1.5)	56 (1.1)	0.037

Peptic ulcer disease	86 (0.2)	62 (0.2)	24 (0.5)	0.054	<0.001	20 (0.4)	24 (0.5)	0.012
Liver disease	1031 (2.5)	672 (1.9)	359 (7.2)	0.258	<0.001	305 (6.1)	359 (7.2)	0.044
Paralysis	126 (0.3)	98 (0.3)	28 (0.6)	0.045	0.001	34 (0.7)	28 (0.6)	0.015
Other neurological disorders	1071 (2.6)	839 (2.4)	232 (4.7)	0.126	<0.001	201 (4.0)	232 (4.7)	0.031
Renal disease	3173 (7.8)	2092 (5.9)	1081 (21.7)	0.473	<0.001	1051 (21.1)	1081 (21.7)	0.015
Cancer	1318 (3.2)	1079 (3.0)	239 (4.8)	0.092	<0.001	229 (4.6)	239 (4.8)	0.009
Hypothyroidism	1335 (3.3)	993 (2.8)	342 (6.9)	0.192	<0.001	347 (7.0)	342 (6.9)	0.004
Coagulation disorders	252 (0.6)	179 (0.5)	73 (1.5)	0.098	<0.001	46 (0.9)	73 (1.5)	0.050
Fluid and electrolyte disorders	1433 (3.5)	1131 (3.2)	302 (6.1)	0.138	0.001	307 (6.2)	302 (6.1)	0.004
Blood loss anaemia	37 (0.1)	28 (0.1)	9 (0.2)	0.028	0.046	8 (0.2)	9 (0.2)	0.005
Deficiency anaemia	371 (0.9)	259 (0.7)	112 (2.3)	0.126	<0.001	71 (1.4)	112 (2.3)	0.061
Alcohol abuse	241 (0.6)	190 (0.5)	51 (1.0)	0.056	<0.001	77 (1.5)	51 (1.0)	0.046
Drug abuse	69 (0.2)	54 (0.2)	15 (0.3)	0.032	0.026	21 (0.4)	15 (0.3)	0.020
Psychosis	151 (0.4)	112 (0.3)	39 (0.8)	0.064	<0.001	30 (0.6)	39 (0.8)	0.022
Depression	1074 (2.6)	826 (2.3)	248 (5.0)	0.143	<0.001	248 (5.0)	248 (5.0)	0.001
Charlson Comorbidity Index								
0	30,361 (74.8)	28,101 (78.9)	2260 (45.5)			2306 (46.4)	2260 (45.5)	
1–2	7484 (18.4)	5703 (16.0)	1781 (35.8)	0.744	<0.001	1802 (36.3)	1781 (35.8)	0.035
3–4	2186 (5.4)	1441 (4.0)	745 (15.0)			694 (14.0)	745 (15.0)	
5+	571 (1.4)	386 (1.1)	185 (3.7)			169 (3.4)	185 (3.7)	

CHF: congestive heart failure, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, PSM: propensity score matched, SMD: standardized mean difference. Pearson's chi-square or Fisher's exact tests were applied to compare diabetes with variables.

3. Results

Table 1 shows the distribution of characteristics and comorbidities of hospitalized COVID-19 patients in unmatched and PSM cohorts. In the unmatched cohort, two-thirds of patients were 60 to 89 years old and 52.4% were men. Common comorbidities were cardiovascular disease (33.1%), hypertension (26%), chronic renal disease (7.8%), COPD (5.6%), and dementia (5.0%). Of the total patients, 18.4% had a CCI score of 1–2, 5.4% had 3–4, and 1.4% had a score of ≥5.

A total of 4971 (12.2%) hospitalized patients had diabetes, 5968 (14.7%) were admitted to ICU, and 6569 (16.2%) died in the hospital. Patients with diabetes were older ($p < 0.001$), included more males (57.5% vs. 51.7%, $p < 0.001$), and had a higher ICU admission rate (18.3% vs. 14.2%, $p < 0.001$) and in-hospital mortality (18.8% vs. 15.8%, $p < 0.001$) than those without diabetes. Among comorbidities, CVD (78.1% vs. 26.9%, $p < 0.001$), COPD (11.5% vs. 4.8%, $p < 0.001$), dementia (9.7% vs. 4.4%, $p < 0.001$), liver disease (7.2% vs. 1.9%, $p < 0.001$), chronic renal disease (21.7% vs. 5.9%, $p < 0.001$), cancer (4.8% vs. 3.0%, $p < 0.001$), hypothyroidism (6.9% vs. 2.8%, $p < 0.001$), fluid and electrolyte disorders (6.1% vs. 3.2%, $p < 0.001$), and depression (5.0% vs. 2.3%, $p < 0.001$) were significantly more prevalent in patients with diabetes than those without diabetes. Patients with diabetes had significantly higher ($p < 0.001$) CCI scores compared to patients without diabetes. In the PSM cohort of 4971 patients with diabetes and 4971 matched patients without diabetes, characteristics and comorbidities were well balanced for 13 variables as indicated by the SMD of <0.10 (Table 1).

Table 2 shows the comparison and logistic regression analysis of ICU admission with diabetes and other variables for both unmatched and PSM cohorts. In the unmatched analysis, patients with diabetes were 36% more likely (OR: 1.36, 95%CI: 1.25–1.47, $p < 0.001$) to be admitted to ICU compared to patients without diabetes. This association was attenuated by more than half (OR: 1.15, 95%CI: 1.04–1.28, $p = 0.009$) in the PSM analysis, nonetheless remaining significant. In addition, males and older patients were more likely to be admitted to ICU in

both unmatched and PSM analyses. The odds of ICU admission were significantly higher in patients having a myocardial infarction, CHF, respiratory disorders, and coagulation disorders compared to those without these comorbidities in both unmatched and PSM analyses. A CCI score of 3–4 showed a significant association with ICU admission in the PSM analysis.

Table 2. Comparison and simple logistic regression analysis of ICU admission with diabetes and other variables in unmatched and propensity score matched cohorts.

Variables	Unmatched Cohort				PSM Cohort	
	No ICU Admission (N = 34,634) n (%)	ICU Admission (N = 5968) n (%)	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Diabetes	4060 (11.7)	911 (15.3)	1.36 (1.25–1.47)	<0.001	1.15 (1.04–1.28)	0.009
Men	17,430 (50.3)	3848 (64.5)	Reference		Reference	
Women	17,204 (49.7)	2120 (35.5)	0.56 (0.53–0.59)	<0.001	0.56 (0.50–0.63)	<0.001
Age groups						
20–29	982 (2.8)	75 (1.3)	Reference		Reference	
30–39	1427 (4.1)	149 (2.5)	1.37 (1.03–1.83)	0.033	0.33 (0.09–1.47)	0.138
40–49	2161 (6.2)	318 (5.3)	1.92 (1.49–2.52)	<0.001	0.60 (0.18–2.39)	0.441
50–59	4456 (12.9)	907 (15.2)	2.66 (2.10–3.43)	<0.001	0.54 (0.17–2.11)	0.348
60–69	5261 (15.2)	1413 (23.7)	3.51 (2.77–4.51)	<0.001	0.65 (0.20–2.54)	0.507
70–79	7838 (22.6)	1826 (30.6)	3.04 (2.41–3.90)	<0.001	0.48 (0.15–1.89)	0.271
80–89	9365 (27.0)	1167 (19.6)	1.63 (1.29–2.09)	<0.001	0.22 (0.07–0.86)	0.032
90+	3144 (9.1)	113 (1.9)	0.47 (0.35–0.64)	<0.001	0.04 (0.01–0.17)	<0.001
Comorbidities						
Myocardial infarction	239 (0.7)	101 (1.7)	2.48 (1.95–3.12)	<0.001	2.27 (1.68–3.04)	<0.001
Cardiac arrhythmias	3612 (10.4)	623 (10.4)	1.00 (0.91–1.09)	0.978	0.93 (0.82–1.05)	0.267
Valvular heart diseases	844 (2.4)	146 (2.5)	1.00 (0.84–1.20)	0.957	0.87 (0.69–1.10)	0.255
Hypertension	8929 (25.8)	1609 (27.0)	1.06 (1.00–1.13)	0.056	1.09 (0.98–1.22)	0.112
Congestive heart failure	1676 (4.8)	335 (5.6)	1.17 (1.04–1.32)	0.012	1.25 (1.08–1.45)	0.004
Peripheral vascular disease	1119 (3.2)	222 (3.7)	1.16 (1.00–1.34)	0.054	1.07 (0.89–1.28)	0.449
Stroke	1024 (3.0)	188 (3.1)	1.07 (0.91–1.25)	0.416	1.01 (0.83–1.22)	0.915
COPD	1854 (5.3)	417 (7.0)	1.33 (1.19–1.48)	<0.001	1.23 (1.05–1.43)	0.010
Pulmonary circulation disorders	436 (1.3)	155 (2.6)	2.09 (1.73–2.51)	<0.001	2.19 (1.65–2.87)	<0.001
Dementia	1975 (5.7)	75 (1.3)	0.21 (0.17–0.26)	<0.001	0.14 (0.09–0.20)	<0.001
Rheumatoid disorders	227 (0.7)	27 (0.5)	0.69 (0.45–1.01)	0.059	0.65 (0.37–1.07)	0.097
Peptic ulcer disease	68 (0.2)	18 (0.3)	1.55 (0.89–2.55)	0.116	1.08 (0.46–2.21)	0.852
Liver disease	848 (2.5)	183 (3.1)	1.26 (1.07–1.48)	0.006	1.15 (0.94–1.40)	0.173
Paralysis	105 (0.3)	21 (0.3)	1.17 (0.71–1.83)	0.524	1.68 (0.92–2.91)	0.091
Other neurological disorders	970 (2.8)	101 (1.7)	0.60 (0.48–0.73)	<0.001	0.56 (0.40–0.75)	<0.001
Renal disease	2741 (7.9)	432 (7.2)	0.91 (0.82–1.01)	0.071	0.81 (0.71–0.93)	0.002
Cancer	1128 (3.3)	190 (3.2)	0.98 (0.83–1.14)	0.774	1.00 (0.78–1.27)	0.999
Hypothyroidism	1157 (3.3)	178 (3.0)	0.89 (0.76–1.04)	0.150	0.93 (0.75–1.14)	0.508
Coagulation disorders	167 (0.5)	85 (1.4)	2.98 (2.29–3.87)	<0.001	4.06 (2.81–5.85)	<0.001
Fluid and electrolyte disorders	1280 (3.7)	153 (2.6)	0.69 (0.58–0.81)	<0.001	0.89 (0.71–1.11)	0.292
Deficiency anemia	328 (0.9)	43 (0.7)	0.76 (0.55–1.04)	0.084	0.72 (0.46–1.09)	0.123
Alcohol abuse	188 (0.5)	53 (0.9)	1.65 (1.20–2.22)	0.002	1.54 (1.01–2.29)	0.046
Psychosis	126 (0.4)	25 (0.4)	1.16 (0.74–1.75)	0.511	1.12 (0.58–1.99)	0.721
Depression	937 (2.7)	137 (2.3)	0.85 (0.70–1.01)	0.065	0.93 (0.72–1.18)	0.552
Charlson Comorbidity Index						
0	25,868 (74.7)	4493 (75.3)	Reference		Reference	
1–2	6383 (18.4)	1101 (18.4)	0.99 (0.92–1.07)	0.851	0.90 (0.80–1.01)	0.083
3–4	1885 (5.4)	301 (5.0)	0.92 (0.81–1.04)	0.188	0.84 (0.72–0.99)	0.035
5+	498 (1.4)	73 (1.2)	0.85 (0.65–1.08)	0.176	0.79 (0.58–1.05)	0.110

CI: confidence interval, COPD: chronic obstructive pulmonary disease, ICU: intensive care unit, OR: odds ratio, PSM: propensity score matched.

Table 3 shows the comparison and logistic regression analysis of in-hospital mortality with diabetes and other variables for both unmatched and PSM cohorts. The unmatched analysis showed 24% higher odds of mortality (OR: 1.24, 95%CI: 1.15–1.34, $p < 0.001$) in people with diabetes compared to those without diabetes. The association of diabetes with in-hospital mortality (OR: 1.08, 95%CI: 0.97–1.19, $p = 0.146$) became insignificant in the PSM analysis. Although hypertension was not well balanced between diabetes and non-diabetes patients, adjusting for it did not change the magnitude of association between diabetes and in-hospital mortality significantly (OR: 1.09, 95%CI: 0.99–1.21, $p = 0.065$). In both unmatched and PSM analysis, the odds of mortality increased with age and CCI score and were significantly higher in males and those admitted to ICU. Patients with myocardial infarction, cardiac arrhythmias, valvular heart disease, congestive heart failure, peripheral artery disease, or stroke had higher odds of mortality ($p < 0.001$) compared to those without these CVDs. Likewise, patients with COPD, pulmonary circulation disorders, dementia, chronic renal disease, cancer, and hypothyroidism had higher odds of mortality than those without these comorbidities.

Table 3. Comparison and simple logistic regression analysis of in-hospital mortality with diabetes and other variables in unmatched and propensity score-matched cohorts.

Variables	Unmatched Cohort				PSM Cohort	
	No In-Hospital Mortality (N = 34,033) n (%)	In-Hospital Mortality (N = 6569) n (%)	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Diabetes	4034 (11.9)	937 (14.3)	1.24 (1.15–1.34)	<0.001	1.08 (0.97–1.19)	0.146
Men	17,582 (51.7)	3696 (56.3)	Reference		Reference	
Women	16,451 (48.3)	2873 (43.7)	0.83 (0.79–0.88)	<0.001	0.84 (0.76–0.94)	<0.001
Age						
20–29	1048 (3.1)	9 (0.1)	Reference		Reference	
30–39	1557 (4.6)	19 (0.3)	1.41 (0.65–3.31)	0.396	0.14 (0.00–5.63)	0.250
40–49	2444 (7.2)	35 (0.5)	1.65 (0.82–3.68)	0.168	0.12 (0.01–3.42)	0.165
50–59	5173 (15.2)	190 (2.9)	4.20 (2.28–8.92)	<0.001	0.46 (0.09–11.60)	0.533
60–69	6093 (17.9)	581 (8.8)	10.90 (5.99–22.90)	<0.001	1.05 (0.20–25.80)	0.965
70–79	8018 (23.6)	1646 (25.1)	23.50 (12.9–49.20)	<0.001	2.23 (0.43–54.80)	0.402
80–89	7727 (22.7)	2805 (42.7)	41.50 (22.9–87.00)	<0.001	3.75 (0.72–92.40)	0.133
90+	1973 (5.8)	1284 (19.5)	74.40 (40.9–157.00)	<0.001	6.80 (1.30–168.00)	0.019
ICU admission	3991 (11.7)	1977 (30.1)	3.24 (3.04–3.45)	<0.001	3.09 (2.75–3.47)	<0.001
Comorbidities						
Myocardial infarction	219 (0.6)	121 (1.8)	2.90 (2.31–3.62)	<0.001	2.27 (1.69–3.03)	<0.001
Cardiac arrhythmias	3160 (9.3)	1075 (16.4)	1.91 (1.77–2.06)	<0.001	1.78 (1.60–1.99)	<0.001
Valvular heart disease	751 (2.2)	239 (3.6)	1.67 (1.44–1.94)	<0.001	1.62 (1.32–1.96)	<0.001
Hypertension	8737 (25.7)	1801 (27.4)	1.09 (1.03–1.16)	0.003	0.75 (0.68–0.84)	<0.001
Congestive heart failure	1326 (3.9)	685 (10.4)	2.87 (2.61–3.16)	<0.001	2.72 (2.38–3.10)	0.000
Peripheral vascular disease	962 (2.8)	379 (5.8)	2.11 (1.86–2.38)	<0.001	1.78 (1.52–2.09)	<0.001
Stroke	887 (2.6)	325 (4.9)	1.95 (1.71–2.21)	<0.001	1.75 (1.47–2.07)	<0.001
COPD	1815 (5.3)	456 (6.9)	1.32 (1.19–1.47)	<0.001	1.20 (1.03–1.40)	0.022
Pulmonary circulation disorders	457 (1.3)	134 (2.0)	1.53 (1.26–1.85)	<0.001	1.67 (1.24–2.21)	0.001
Dementia	1344 (3.9)	706 (10.7)	2.93 (2.66–3.22)	<0.001	2.32 (2.00–2.68)	<0.001
Rheumatoid disease	205 (0.6)	49 (0.7)	1.24 (0.90–1.68)	0.183	1.37 (0.90–2.03)	0.140
Peptic ulcer disease	66 (0.2)	20 (0.3)	1.58 (0.93–2.56)	0.087	1.33 (0.62–2.61)	0.443
Liver disease	847 (2.5)	184 (2.8)	1.13 (0.96–1.32)	0.144	1.03 (0.84–1.26)	0.782

Paralysis	100 (0.3)	26 (0.4)	1.35 (0.86–2.06)	0.183	0.77 (0.35–1.49)	0.456
Other neurological disorders	774 (2.3)	297 (4.5)	2.04 (1.77–2.33)	<0.001	2.13 (1.72–2.62)	<0.001
Renal disease	2243 (6.6)	930 (14.2)	2.34 (2.15–2.54)	<0.001	2.28 (2.04–2.55)	<0.001
Cancer	965 (2.8)	353 (5.4)	1.95 (1.72–2.20)	<0.001	1.86 (1.51–2.28)	<0.001
Hypothyroidism	1171 (3.4)	164 (2.5)	0.72 (0.61–0.85)	<0.001	0.60 (0.47–0.76)	<0.001
Coagulation disorders	193 (0.6)	59 (0.9)	1.59 (1.18–2.12)	0.003	1.66 (1.09–2.47)	0.020
Fluid and electrolyte disorders	1172 (3.4)	261 (4.0)	1.16 (1.01–1.33)	0.036	1.19 (0.97–1.45)	0.094
Deficiency anaemia	307 (0.9)	64 (1.0)	1.08 (0.82–1.41)	0.567	0.84 (0.55–1.24)	0.394
Alcohol abuse	177 (0.5)	64 (1.0)	1.88 (1.40–2.50)	<0.001	1.44 (0.94–2.14)	0.090
Psychosis	125 (0.4)	26 (0.4)	1.08 (0.69–1.63)	0.714	1.15 (0.61–2.01)	0.651
Depression	907 (2.7)	167 (2.5)	0.95 (0.80–1.12)	0.575	0.84 (0.65–1.07)	0.161
Charlson Comorbidity Index						
0	26,400 (77.6)	3961 (60.3)	Reference		Reference	
1–2	5752 (16.9)	1732 (26.4)	2.01 (1.88–2.14)	<0.001	2.47 (2.18–2.80)	<0.001
3–4	1491 (4.4)	695 (10.6)	3.11 (2.82–3.42)	<0.001	3.99 (3.44–4.62)	<0.001
5+	390 (1.1)	181 (2.76)	3.09 (2.58–3.70)	<0.001	4.40 (3.45–5.59)	<0.001

CI: confidence interval, COPD: chronic obstructive pulmonary disease, OR: odds ratio, PSM: propensity score matched.

4. Discussion

This countrywide retrospective study examined the impact of diabetes on the severity of the infection and in-hospital mortality in patients hospitalized for COVID-19. The analysis showed that hospitalized COVID-19 patients with diabetes were older and had a higher burden of multimorbidity and CCI score compared to those without diabetes. Patients with diabetes were more likely to have severe COVID-19 disease than those without diabetes as evidenced by higher odds of ICU admission even after propensity matching of characteristics, comorbidities, and the CCI score. However, diabetes was not independently associated with in-hospital mortality after matching diabetes and non-diabetes cohorts. While old age, male sex, and comorbidities were significantly associated with both outcomes in unmatched and matched analyses.

This study adopted the PSM method to assess the independent impact of diabetes on the severity of COVID-19 and in-hospital mortality. The association of diabetes with mortality was attenuated threefold i.e., from 24% in the unmatched cohort to 8% in the PSM cohort. These findings suggest that diabetes has no independent impact on in-hospital mortality in COVID-19 patients. Instead, advanced age, sex, and multimorbidity are responsible for the previously shown association between diabetes and in-hospital mortality. Our findings are consistent with a previous study that employed the PSM analysis and found no significant association between diabetes and in-hospital mortality in French patients hospitalized for COVID-19 [14]. In contrast, most studies have reported a significant positive association between diabetes and COVID-19 related mortality. For instance, a recent meta-analysis has demonstrated a significant association between diabetes and COVID-19 mortality that attenuated after adjusting for age and comorbidities [18]. Another meta-analysis of 31,067 patients has also reported a higher COVID-19 related mortality in patients with diabetes [19]. Similarly, a large study in England reported 80% higher odds of COVID-19 deaths in people with type 2 diabetes than those without diabetes [20]. Likewise, two small studies from China found diabetes to be a significant risk factor of mortality in COVID-19 patients even after adjusting for comorbidities and laboratory markers [21,22]. Even though previous studies have demonstrated a significant association between diabetes and COVID-19 mortality, most studies and those included in meta-analyses have not adjusted for various significant comorbidities and risk factors.

These limitations further support our finding regarding the underlying impact of age, sex, and multimorbidity on COVID-19 mortality in patients with diabetes.

In this study, patients with diabetes were 36% more likely to have severe COVID-19 disease as represented by ICU admission in the unmatched analysis that decreased to 15% in the PSM analysis but remained significant. These findings are consistent with previous studies; however, the direct comparison across studies is not possible because of differences in defining criteria of the severity of COVID-19 disease and statistical analysis methods across studies. Moreover, the degree of association between diabetes and the severity of COVID-19 disease in our study is much lower than in other studies. For instance, meta-analyses performed in different phases of the COVID-19 pandemic have reported the pooled odds of developing severe COVID-19 disease ranging from 1.58 to 2.75 in people with diabetes compared to non-diabetes [23–26]. The vast heterogeneity in the degree of association between diabetes and COVID-19 severity is primarily due to different adjustment factors like age, obesity, smoking, and comorbidities considered by studies [24,27]. Moreover, previous studies have shown that glucose-lowering medication might be associated with COVID-19 outcomes [28]. However, we could not adjust for medication in our study due to the unavailability of treatment regimen information. Of note, dementia was associated with in-hospital mortality, while it was significantly associated with lower ICU admission. We believe that this association is rather due to a selection bias of patients being admitted to ICU than a biological association.

Our findings must be interpreted with some limitations. As common in healthcare data, there is a possibility of miscoding or under-coding of diagnoses in this database. Therefore, diabetes was not classified into subtypes because of the uncertainty in the ICD coding accuracy of this diagnosis. Moreover, laboratory measurements of glucose and other biochemical parameters were not available for all patients and therefore not included in the analysis. This limitation might have resulted in residual confounding between diabetes and COVID-19 outcomes. Furthermore, in-hospital mortality was defined as death from any underlying cause, which might have captured deaths resulting from causes other than COVID-19. However, considering that this database holds data only on patients with COVID-19, the likelihood of including non-COVID-19-related deaths is low. In addition, we only analyzed in-hospital mortality in this study, however previous studies have shown that the pandemic might impact overall mortality in people with diabetes as well [29]. Last, our cohort comprised Austrian citizens only, hence the findings might not be generalized to other populations with different ethnicities.

5. Conclusions

This countrywide retrospective cohort analysis of patients hospitalized for COVID-19 found that people with diabetes had an advanced age and a higher burden of multimorbidity compared to those without diabetes. Consistent with the existing research, hospitalized patients with diabetes were significantly more likely to suffer from severe COVID-19 illness compared to those without diabetes; whereas, comorbidities and old age rather than diabetes per se were responsible for the increased likelihood of COVID-19 related in-hospital mortality. This study has confirmed that advanced age and comorbidities play a major role in both COVID-19 disease severity and death even in patients with diabetes and hence should be considered in risk stratification, management of patients, and national vaccination strategies.

Author Contributions: Conceptualization, F.A. (Faisal Aziz), H.S. (Harald Sourij) and S.K.; methodology, F.A. (Faisal Aziz), H.S. (Harald Sourij) and S.K.; software, F.A. (Faisal Aziz); validation, H.S. (Harald Sourij) and S.K.; formal analysis, F.A. (Faisal Aziz); investigation, H.S. (Harald Sourij); resources, H.S. (Harald Sourij); data curation, F.A. (Faisal Aziz); writing—original draft preparation, F.A. (Faisal Aziz), H.S. (Harald Sourij), S.K., F.A. (Felix Aberer), and N.T.; writing—review and editing, All co-authors; visualization, F.A. (Faisal Aziz) and H.S. (Harald Sourij); supervision, H.S. (Harald Sourij) and S.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was reviewed by the ethics committee of the Medical University of Graz, Graz, Austria (EK 32-355 ex 19/20) and conformed to the 1964 declaration of Helsinki and guidelines of the International Conference on Harmonization for Good Clinical Practice (ICH GCP E6 guidelines).

Informed Consent Statement: This study was a retrospective analysis of anonymized data, therefore, no informed consent was obtained from the patients.

Data Availability Statement: Data included in this study is a property of the Austrian National Public Health Institute (Gesundheit Österreich GmbH). Further information regarding data access is available at: https://datenplattform-covid.goeg.at/english.

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Conflicts of Interest: All authors do not have any conflict of interest to declare.

Appendix A

Table A1. ICD-10 codes of comorbidities.

Comorbidities	ICD-10 Codes
Hypertension	I10.x, I11.x–I13.x, I15.x
Myocardial infarction/Ischaemic heart disease	I21.x, I22.x, I25.x
Congestive heart failure	I09.9, I11.0, I13.0, I13.2, I25.5, I42.0, I42.5–I42.9, I43.x, I50.x, P29.0
Cardiac arrhythmias	I44.1–I44.3, I45.6, I45.9, I47.x–I49.x, R00.0, R00.1, R00.8, T82.1, Z45.0, Z95.0
Valvular disease	A52.0, I05.x–I08.x, I09.1, I09.8, I34.x–I39.x, Q23.0–Q23.3, Z95.2–Z95.4
Peripheral vascular disease	I70.x, I71.x, I73.1, I73.8, I73.9, I77.1, I79.0, I79.2, K55.1, K55.8, K55.9, Z95.8, Z95.9
Cerebrovascular disease	G45.x, G46.x, H34.0, I60.x–I69.x
Dementia	F00.x–F03.x, F05.1, G30.x, G31.1
Chronic obstructive pulmonary disease	I27.8, I27.9, J40.x–J47.x, J60.x–J67.x, J68.4, J70.1, J70.3
Pulmonary circulation disorders	I26.x, I27.x, I28.0, I28.8, I28.9
Rheumatic disease	M05.x, M06.x, M31.5, M32.x–M34.x, M35.1, M35.3, M36.0
Peptic ulcer disease	K25.x–K28.x
Liver disease	B18.x, K70.0–K70.3, K70.9, K71.3–K71.5, K71.7, K73.x, K74.x, K76.0, K76.2–K76.4, K76.8, K76.9, Z94.4, I85.0, I85.9, I86.4, I98.2, K70.4, K71.1, K72.1, K72.9, K76.5, K76.6, K76.7
Diabetes	E10.x, E11.x, E12.x, E13.x, E14.x
Paralysis	G04.1, G11.4, G80.1, G80.2, G81.x, G82.x, G83.0–G83.4, G83.9

Other neurological disorders	G10.x–G13.x, G20.x–G22.x, G25.4, G25.5, G31.2, G31.8, G31.9, G32.x, G35.x–G37.x, G40.x, G41.x, G93.1, G93.4, R47.0, R56.x
Hypothyroidism	E00.x–E03.x, E89.0
Renal disease	I12.0, I13.1, N03.2–N03.7, N05.2–N05.7, N18.x, N19.x, N25.0, Z49.0–Z49.2, Z94.0, Z99.2
Any malignancy, except malignant neoplasm of skin	C00.x – C26.x, C30.x – C34.x, C37.x – C41.x, C43.x, C45.x – C58.x, C60.x – C76.x, C81.x – C85.x, C88.x, C90.x – C97.x
Metastatic solid tumour	C77.x–C80.x
AIDS/HIV	B20.x–B22.x, B24.x
Rheumatoid arthritis/collagen vascular diseases	L94.0, L94.1, L94.3, M05.x, M06.x, M08.x, M12.0, M12.3, M30.x, M31.0–M31.3, M32.x–M35.x, M45.x, M46.1, M46.8, M46.9
Coagulopathy	D65–D68.x, D69.1, D69.3–D69.6
Fluid and electrolyte disorders	E22.2, E86.x, E87.x
Blood loss anaemia	D50.0
Deficiency anaemia	D50.8, D50.9, D51.x–D53.x
Alcohol abuse	F10, E52, G62.1, I42.6, K29.2, K70.0, K70.3, K70.9, T51.x, Z50.2, Z71.4, Z72.1
Drug abuse	F11.x–F16.x, F18.x, F19.x, Z71.5, Z72.2
Psychoses	F20.x, F22.x–F25.x, F28.x, F29.x, F30.2, F31.2, F31.5
Depression	F20.4, F31.3–F31.5, F32.x, F33.x, F34.1, F41.2, F43.2

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7.3. Appendix 3

viruses

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Article

Simplified Acute Physiology Score 3 Performance in Austrian COVID-19 Patients Admitted to Intensive Care Units with and without Diabetes

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Abstract: This study evaluated and compared the performance of simplified acute physiology score 3 (SAPS 3) for predicting in-hospital mortality in COVID-19 patients admitted to intensive care units (ICUs) with and without diabetes in Austria. The Austrian national public health institute (GÖG) data of COVID-19 patients admitted to ICUs (n = 5850) were analyzed. Three versions of SAPS 3 were used: standard equation, Central European equation, and Austrian equation customized for COVID-19 patients. The observed in-hospital mortality was 38.9%, 42.9%, and 37.3% in all, diabetes, and non-diabetes patients, respectively. The overall C-statistics was 0.69 with an insignificant (p = 0.193) difference between diabetes (0.70) and non-diabetes (0.68) patients. The Brier score was > 0.20 for all SAPS 3 equations in all cohorts. Calibration was unsatisfactory for both standard and Central European equations in all cohorts, whereas it was satisfactory for the Austrian equation in diabetes patients only. The SAPS 3 score demonstrated low discrimination and accuracy in Austrian COVID-19 patients, with an insignificant difference between diabetes and non-diabetes. All equations were miscalibrated particularly in non-diabetes patients, while the Austrian equation showed satisfactory calibration in diabetes patients only. Both uncalibrated and calibrated versions of SAPS 3 should be used with caution in COVID-19 patients.

Keywords: SAPS 3; simplified acute physiology score; diabetes; intensive care unit; mortality; COVID-19; SARS-CoV-2

1. Introduction

Coronavirus disease (COVID-19) has caused a devastating pandemic with a high hospitalization rate and mortality. As of 6 December 2021, more than 272 million cases of COVID-19 and more than 5 million deaths have been reported worldwide [1]. This health crisis has severely challenged the capacity of healthcare systems to treat hospitalized

and critically ill COVID-19 patients [2]. In such a situation, prognostic scores may offer a cost-effective and practical strategy to prioritize and allocate health resources, guide patient management, and evaluate the effectiveness of therapeutic interventions in intensive care units (ICUs) [3].

Numerous scoring systems are routinely utilized in ICUs to evaluate the quality of care and predict the prognosis of patients [3]. However, these scores need to be updated regularly to adjust for diagnostic and therapeutic advances in the ICU practice and changes in disease patterns [3]. The simplified acute physiology score version 3 (SAPS 3) is one of the most widely used scores in the ICU and has been extensively validated in Europe and other regions. The SAPS 3 incorporates various ICU- and patient-related factors for assessing disease severity and predicting in-hospital mortality [4].

Despite the obvious benefits of prognostication scores, validation is required in COVID-19 patients before their application in this population. In this regard, a few studies have evaluated the performance of various scores including SAPS 3 in COVID-19 patients albeit with contradictory findings. The SAPS 3 has overestimated the mortality in high-risk Brazilian patients suffering from COVID-19, whereas it has significantly underpredicted the mortality in Austrian COVID-19 patients. In addition, calibration of SAPS 3 was inadequate in both Brazilian and Austrian COVID-19 patients [5,6]. These conflicting results warrant more validation studies of SAPS 3 in COVID-19 cohorts. In addition, as people with diabetes are regarded as a high-risk group for COVID-19 morbidity and mortality, no previous study has compared the performance of SAPS 3 in COVID-19 patients with and without diabetes. In this study, we evaluated the performance of SAPS 3 for predicting in-hospital mortality in a countrywide cohort of COVID-19 patients admitted to ICUs in Austria. In addition, we compared its predictive performance between patients with and without diabetes.

2. Methods

2.1. Study Design and Data Source

The "Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD)" checklist was used for reporting this study [7]. This study retrospectively analyzed the cohort of patients with and without diabetes mellitus admitted to ICUs following primary or secondary diagnosis of SARS-CoV-2 infection from March 2020 to March 2021 in Austria. These data are collected and maintained by the "Data platform COVID-19" commissioned by the Austrian National Public Health Institute (Gesundheit Österreich GmbH, Vienna, Austria). This platform gathers nationally representative countrywide epidemiological and clinical data of COVID-19 patients to provide updated evidence on SARS-CoV-2 infection in Austria. The details of this data platform can be accessed at: https://datenplattform-covid.goeg.at/english (accessed on 3 May 2021).

2.2. Data Extraction

For this study, two anonymized datasets were received from the Austrian data platform: (1) hospital data that comprised variables on demographic characteristics, comorbidities, ICU stay, and in-hospital mortality and (2) SAPS 3 data that comprised variables for calculating SAPS 3 score and the number of readmissions in the ICU. Data of patients admitted to ICU were extracted from the hospital data and then matched and merged with the SAPS 3 data after removing readmissions. Afterwards, patients aged less than 20 years were removed from the merged data, as only adults were considered in the study. A total of 5850 patients were included in the final analysis (Figure 1).

2.3. Study Variables

The outcome variable was in-hospital mortality, which was defined as death occurring in the hospital following hospitalization for primary or secondary SARS-CoV-2 infection or discharged alive from the hospital. Diabetes was recorded in the database as a comorbidity

(insulin and non-insulin dependent diabetes) for the SAPS 3 and as per International Classification of Disease (ICD) version 10 codes (E10, E11, E12, E13, E14).

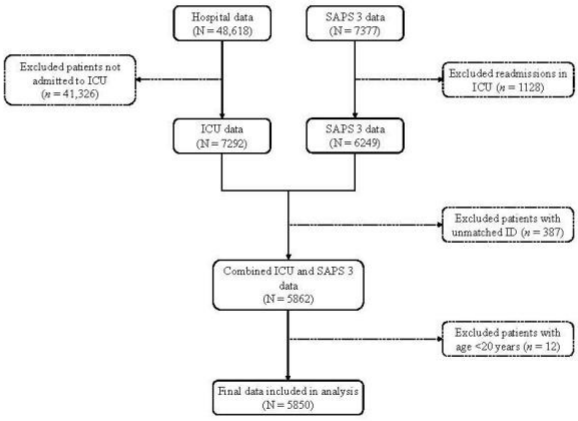

Figure 1. Flow diagram of data extraction. ICU, intensive care unit; SAPS 3, simplified acute physiology score 3.

The SAPS 3 score consists of 20 variables that were recorded at the time of ICU admission. These variables were classified as patient characteristics, reasons for ICU admission, and acute physiological disruptions. Patient characteristics included age deciles (20–90+ years), gender, previous health status, comorbidities, intra-hospital location before ICU, length of stay in the hospital before ICU admission, and major therapeutic interventions before ICU admission. Reasons for ICU admission included health conditions, status and site of surgery, and the presence of infection at ICU admission. Acute physiological disruptions were measured in terms of vital signs, neurological status, serum creatinine, leukocytes, platelets, blood pH, partial pressure of oxygen (PaO_2), and a fraction of inspired oxygen (FiO_2). The detailed information regarding the calculation of the SAPS 3 score is published elsewhere [4].

2.4. Statistical Analysis

2.4.1. Summary Statistics

Data were received in Microsoft Excel and analyzed in R version 1.4.1 and Stata version 17.0 (Stata Corp, Houston, TX, USA). Missing values of SAPS 3 variables were replaced with either reference or normal categories as recommended in the SAPS 3 publication. Continuous variables were reported as mean ± standard deviation (SD) or median and interquartile range (IQR) if not normally distributed. Categorical variables were reported as frequencies with corresponding percentages (%).

2.4.2. Calculation of SAPS 3 Score and Predicted in-Hospital Mortality

The SAPS 3 score was calculated only for the first episode of ICU admission using the variables and algorithm recommended in the original publication [4]. The predicted in-hospital mortality was estimated from the SAPS 3 score using three logit regression equations: (1) standard equation (Logit = −32.6659 + ln [SAPS 3 score + 20.5958] × 7.3068); (2) Central European equation (Logit = −36.0877 + ln [SAPS 3 score + 22.2655] × 7.9867); and (3) recently published Austrian equation recalibrated for COVID-19 patients (Logit = −14.451 + ln [SAPS 3 score + −12.092] × 3.666) [4,6]. In addition, the standardized mortality ratio (SMR) was estimated by dividing the observed mortality rate with the predicted mortality rate with corresponding 95% confidence intervals (CI) to test the

uniformity of fit. The value of SMR < 1 indicates overestimation, while >1 indicates an underestimation of the outcome.

2.4.3. Assessment of Predictive Performance of SAPS 3

The predictive performance of each SAPS 3 equation was assessed in terms of discrimination, calibration, and accuracy. Discrimination was assessed by estimating the area under the receiver operating characteristics curve (AUC) or C-statistic with corresponding 95% CI. The AUC was compared between patients with and without diabetes using the DeLong test, and a *p*-value of <0.05 was chosen to determine statistical significance. The Youden index was estimated to select the optimal cut-off value of SAPS 3 score for the overall, diabetes, and non-diabetes cohorts. The identified cut-off values were then used to calculate sensitivity, specificity, and predictive values of SAPS 3 score.

Calibration was assessed by comparing the predicted probability against the observed probability using the Hosmer–Lemeshow (H-L) goodness-of-fit test and the calibration plot. In the H-L test, the *p*-value > 0.05 indicates a good fit. In the calibration plot, the calibration slope close to 1 indicates good calibration, the calibration intercept (calibration in the large (CITL)) close to 0 indicates good calibration, and the alignment of calibration lowess curve with the reference line indicates good calibration. Accuracy of the SAPS 3 in predicting in-hospital mortality was assessed using the Brier score. The Brier score ranges from 0 to 0.25, with 0 indicating perfect accuracy and 0.25 indicating non-informative accuracy.

2.5. Ethical Considerations

This study was approved by the Ethics Committee of the Medical University of Graz, Graz, Austria (ethics number 32-355 ex 19/20). This study followed the guidelines of good clinical practice and the Declaration of Helsinki 1964. No consent forms were obtained from the study participants, as it was a retrospective analysis of pseudonymized data.

3. Results

3.1. Characteristics of Patients

Table 1 shows the distribution of characteristics, SAPS 3 variables, and SAPS 3 score in COVID-19 patients admitted to ICU in all, diabetes, and non-diabetes patients. Of the 5850 patients admitted to ICU, 1667 (28.50%) had diabetes. Most patients were males (66.07%) and aged above 60 years. The mean ± SD SAPS 3 score was 57.39 ± 13.18 in the overall cohort and was significantly higher in patients with diabetes than those without diabetes (58.78 ± 12.92 vs. 56.84 ± 13.23, $p < 0.001$).

Table 1. Characteristics of COVID-19 patients admitted to intensive care units, overall, and by diabetes status.

Variable	All	Diabetes		*p*-Value
		Yes	No	
All, *n* (%)	5850	1667 (28.50)	4183 (71.50)	–
Sex, *n* (%)				
Female	1985 (33.93)	541 (32.45)	1444 (34.52)	0.132
Male	3865 (66.07)	1126 (67.55)	2739 (65.48)	
Age, years, *n* (%)				
<40	215 (3.68)	22 (1.32)	193 (4.61)	<0.001
40–59	1258 (21.50)	330 (19.80)	928 (22.19)	
60–69	1471 (25.15)	457 (27.41)	1014 (24.24)	
70–74	906 (15.49)	312 (18.72)	594 (14.20)	
75–79	895 (15.30)	259 (15.54)	636 (15.20)	
≥80	1105 (18.89)	287 (17.22)	818 (19.56)	
Stay in hospital before ICU admission, days, *n* (%)				

Table 1. *Cont.*

Variable	All	Diabetes		*p*-Value
		Yes	No	
<14	4437 (75.85)	1254 (75.22)	3183 (76.09)	0.781
14–27	912 (15.59)	267 (16.02)	645 (15.42)	
≥28	501 (8.56)	146 (8.76)	355 (8.49)	
Intra-hospital location before ICU admission, *n* (%)				0.017
Operative room	334 (5.71)	70 (4.20)	264 (6.31)	
Emergency room	920 (15.73)	265 (15.90)	655 (15.66)	
Other ICU	1160 (19.83)	344 (20.64)	816 (19.51)	
Hospital wards	3436 (58.74)	988 (59.27)	2448 (58.52)	
Comorbidities				
Cancer therapy, *n* (%)	480 (8.21)	154 (9.24)	326 (7.79)	0.069
Congestive heart failure, NYHA IV, *n* (%)	131 (2.24)	49 (2.94)	82 (1.96)	0.022
Hematological cancer, *n* (%)	148 (2.53)	29 (1.74)	119 (2.84)	0.015
Cirrhosis, *n* (%)	86 (1.47)	25 (1.50)	61 (1.46)	0.905
AIDS, *n* (%)	3 (0.05)	3 (0.18)	0 (0.00)	0.023
Cancer with metastasis, *n* (%)	114 (1.95)	16 (0.96)	98 (2.34)	0.001
Vasoactive drugs before ICU admission, *n* (%)	916 (15.66)	246 (14.76)	670 (16.02)	0.231
Reasons for ICU admission				0.043
Cardiovascular, *n* (%)				
Arrhythmia	112 (1.91)	20 (1.20)	92 (2.20)	
All others	5576 (95.32)	1601 (96.04)	3975 (95.03)	
Hypovolemic shock	32 (0.55)	6 (0.36)	26 (0.62)	
Septic, anaphylactic, undefined, and mixed shock	130 (2.22)	40 (2.40)	90 (2.15)	
Hepatic, *n* (%)				0.420
All other	5831 (99.68)	1660 (99.58)	4171 (99.71)	
Liver failure	19 (0.32)	7 (0.42)	12 (0.29)	
Digestive, *n* (%)				0.260
All others	5783 (98.85)	1653 (99.16)	4130 (98.73)	
Acute abdomen, other	54 (0.92)	10 (0.60)	44 (1.05)	
Severe pancreatitis	13 (0.22)	4 (0.24)	9 (0.22)	
Neurologic, *n* (%)				0.007
Seizures	19 (0.32)	1 (0.06)	18 (0.43)	
All others	5482 (93.71)	1562 (93.70)	3920 (93.71)	
Coma, stupor, obtund patient, agitation, vigilance disturbances, confusion, delirium	248 (5.24)	85 (5.10)	163 (3.90)	
Focal neurological deficit	71 (1.21)	12 (0.72)	59 (1.41)	
Intracranial mass effect	30 (0.51)	7 (0.42)	23 (0.55)	
Surgical status at ICU admission, *n* (%)				<0.001
No surgery	5251 (89.76)	1552 (93.10)	3699 (88.43)	
Scheduled surgery	294 (5.03)	64 (3.84)	230 (5.50)	
Emergency surgery	305 (5.21)	51 (3.06)	254 (6.07)	
Anatomical site of surgery, *n* (%)				0.169
Transplant surgery	1 (0.02)	0 (0.00)	1 (0.02)	
Trauma	51 (0.87)	11 (0.66)	40 (0.96)	
Cardiac surgery	23 (0.39)	6 (0.36)	17 (0.41)	
All others	5746 (98.22)	1647 (98.80)	4099 (97.99)	
Neurosurgery	29 (0.50)	3 (0.18)	26 (0.62)	

Table 1. *Cont.*

Variable	All	Diabetes		*p*-Value
		Yes	No	
GCS score, median (IQR)	15 (1)	15 (1)	15 (1)	0.016
Mean ± SD	13.40 ± 3.51	13.34 ± 3.50	13.43 ± 3.51	
Total bilirubin, mg/dL, median (IQR)	0.60 (0.50)	0.50 (0.40)	0.60 (0.50)	<0.001
Body temperature, °C, mean ± SD	37.27 ± 1.30	37.31 ± 1.29	37.26 ± 1.21	0.192
Creatinine, mg/dL, median (IQR)	1.00 (0.60)	1.10 (0.90)	1.00 (0.60)	<0.001
Heart rate, bpm, mean ± SD	90 ± 30	93 ± 30	90 ± 29	<0.001
Leukocytes, G/L, median (IQR)	9.40 (6.40)	9.40 (6.20)	9.40 (6.50)	0.775
Hydrogen ion, pH, median (IQR)	7.42 (0.12)	7.41 (0.13)	7.42 (0.11)	<0.001
Platelets, G/L, median (IQR)	218.00 (122.00)	224.00 (123.50)	215.50 (123.00)	0.043
Systolic blood pressure, mmHg, mean ± SD	116.12 ± 31.27	116.68 ± 32.35	115.89 ± 30.81	0.417
PaO_2, mmHg, median (IQR)	69 (27)	68 (26)	69 (28)	0.052
FiO_2, %, median (IQR)	60 (40)	65 (30)	60 (40)	<0.001
SAPS 3 score, mean ± SD	57.39 ± 13.18	58.78 ± 12.92	56.84 ± 13.23	<0.001

GCS, Glasgow Coma Scale; ICU, intensive care unit; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of oxygen in arterial blood; SAPS 3, simplified acute physiology score 3.

In reasons for admission, the category "all others" in each system include reasons that are either not related to that particular system or those not falling in specified categories within that system.

Pearson's chi-square or Fisher's exact test were applied to compare qualitative variables with diabetes status. Two sample *t*-tests or Wilcoxon rank sum tests were applied to compare quantitative variables with diabetes status.

3.2. *Observed In-Hospital Mortality and Its Comparison with Variables*

Table 2 shows that the overall observed in-hospital mortality was 38.91%, and it was significantly higher in patients with diabetes (42.95% vs. 37.29%, $p < 0.001$) compared to those without diabetes. Patients who died in the hospital had significantly higher mean ± SD SAPS 3 scores compared to those who were alive in all (62.57 ± 12.86 vs. 54.10 ± 12.29, $p < 0.001$), diabetes (63.96 ± 13.15 vs. 54.87 ± 11.28, $p < 0.001$), and non-diabetes patients each (61.92 ± 12.68 vs. 53.82 ± 12.62, $p < 0.001$).

In reasons for admission, the category "all others: in each system include reasons that are either not related to that particular system or those not falling in specified categories within that system.

Pearson's chi-square or Fisher's exact test were applied to compare qualitative variables with in-hospital mortality status. Two sample *t*-tests or Wilcoxon rank sum tests were applied to compare quantitative variables with in-hospital mortality status.

3.3. *Predicted In-Hospital Mortality and Standardized Mortality Ratio*

Table 3 shows that the mean predicted mortality estimated by the SAPS 3 standard equation in all patients was 32.47 ± 21.69, Central European equation was 28.05 ± 21.43, and Austrian equation was 37.86 ± 20.56. The predicted mortality was significantly higher in patients with diabetes compared to those without diabetes for standard (34.56 ± 21.62 vs. 31.63 ± 21.66, $p < 0.001$), Central European (30.02 ± 21.56 vs. 27.28 ± 21.33, $p < 0.001$), and Austrian equations each (40.03 ± 20.15 vs. 37.00 ± 20.66, $p < 0.001$). The SMR value > 1 with their corresponding CIs for both standard and Central European equations indicated that these equations significantly underestimated the in-hospital mortality in all three patient

populations i.e., all, diabetes, and non-diabetes patients. The Austrian equation concorded well with the observed mortality in the overall and non-diabetes cohorts, whereas it slightly underpredicted the mortality in patients with diabetes.

Table 2. Comparison of observed in-hospital mortality in COVID-19 patients admitted to intensive care unit with diabetes and SAPS 3 variables.

Characteristic	In-Hospital Mortality		*p*-Value
	Yes	No	
All, *n* (%)	2276 (38.91)	3574 (61.09)	–
Diabetes, *n* (%)			
No	1560 (37.29)	2623 (62.71)	<0.001
Yes	716 (42.95)	951 (57.05)	
Sex, *n* (%)			
Female	742 (37.38)	1243 (62.62)	0.086
Male	1534 (39.69)	2331 (60.31)	
Age, years, *n* (%)			
<40	22 (10.23)	193 (89.77)	
40–59	219 (17.41)	1039 (82.59)	
60–69	496 (33.72)	975 (66.28)	<0.001
70–74	401 (44.26)	505 (55.74)	
75–79	467 (52.18)	428 (47.82)	
≥80	671 (60.72)	434 (39.28)	
Stay in hospital before ICU admission, days, *n* (%)			
<14	2009 (45.28)	2428 (54.72)	
14–27	197 (21.60)	715 (78.40)	<0.001
≥28	70 (13.97)	431 (86.03)	
Intra-hospital location before ICU admission, *n* (%)			
Operative room	64 (19.16)	270 (80.84)	
Emergency room	326 (35.43)	594 (64.57)	<0.001
Other ICU	479 (41.29)	681 (58.71)	
Hospital wards	1407 (40.95)	2029 (59.05)	
Comorbidities			
Cancer therapy, *n* (%)	247 (51.46)	233 (48.54)	<0.001
Congestive heart failure, NYHA IV, *n* (%)	84 (64.12)	47 (35.88)	<0.001
Hematological cancer, *n* (%)	72 (48.65)	76 (51.35)	0.003
Cirrhosis, *n* (%)	52 (60.47)	34 (39.53)	0.001
AIDS, *n* (%)	0 (0.00)	3 (100.00)	0.167
Cancer with metastasis, *n* (%)	60 (52.63)	54 (47.37)	0.002
Vasoactive drugs before ICU admission, *n* (%)	429 (46.83)	487 (53.17)	<0.001
Reasons for ICU admission			
Cardiovascular, *n* (%)			
Arrhythmia	35 (31.25)	77 (78.75)	
All others	2155 (38.35)	3421 (61.35)	<0.001
Hypovolemic shock	15 (46.88)	17 (53.12)	
Septic, anaphylactic, undefined, and mixed shock	71 (54.62)	59 (45.38)	
Hepatic, *n* (%)			
All other	1970 (33.78)	3861 (66.22)	<0.001
Liver failure	15 (78.95)	4 (21.05)	
Digestive, *n* (%)			
All others	2262 (39.11)	3521 (60.89)	
Acute abdomen, other	9 (16.67)	45 (83.33)	0.003
Severe pancreatitis	5 (38.46)	8 (61.54)	

Table 2. *Cont.*

Characteristic	In-Hospital Mortality		*p*-Value
	Yes	No	
Neurologic, *n* (%)			
Seizures	6 (31.58)	13 (68.62)	0.045
All others	2115 (38.58)	3367 (61.42)	
Coma, stupor, obtunded patient, agitation, vigilance disturbances, confusion, delirium	119 (47.98)	129 (52.02)	
Focal neurological deficit	25 (35.21)	46 (64.79)	
Intracranial mass effect	11 (36.67)	19 (63.33)	
Surgical status at ICU admission, *n* (%)			
No surgery	2155 (41.04)	3096 (58.96)	<0.001
Scheduled surgery	47 (15.99)	247 (84.01)	
Emergency surgery	74 (24.26)	231 (75.74)	
Anatomical site of surgery, *n* (%)			
Transplant surgery	1 (100.00)	0 (0.00)	<0.001
Trauma	14 (27.45)	37 (72.55)	
Cardiac surgery	0 (0.00)	23 (100.00)	
All others	2255 (39.24)	3491 (60.76)	
Neurosurgery	6 (20.69)	23 (79.31)	
GCS score, median (IQR)	15 (2)	15 (0)	<0.001
Mean ± SD	12.72 ± 3.99	13.83 ± 3.08	
Total bilirubin, mg/dL, median (IQR)	0.60 (0.50)	0.60 (0.40)	0.001
Body temperature, °C, mean ± SD	37.26 ± 1.29	37.29 ± 1.20	0.471
Creatinine, mg/dL, median (IQR)	1.19 (0.80)	0.90 (0.50)	<0.001
Heart rate, bpm, mean ± SD	97 ± 26	93 ± 23	<0.001
Leukocytes, G/L, median (IQR)	9.87 (7.10)	9.00 (5.80)	<0.001
Hydrogen ion, pH, median (IQR)	7.40 (0.14)	7.43 (0.09)	<0.001
Platelets, G/L, median (IQR)	202.00 (117.00)	229.00 (123.00)	<0.001
Systolic blood pressure, mmHg, mean ± SD	112.57 ± 31.93	118.43 ± 30.62	<0.001
PaO_2, mmHg, median (IQR)	66 (24)	70 (29)	<0.001
FiO_2, %, median (IQR)	70 (40)	55 (40)	<0.001
SAPS 3 score			
All patients, mean ± SD	62.57 ± 12.86	54.10 ± 12.29	<0.001
Diabetes, mean ± SD	63.96 ± 13.15	54.87 ± 11.28	<0.001
No diabetes, mean ± SD	61.92 ± 12.68	53.82 ± 12.62	<0.001

GCS, Glasgow Coma Scale; ICU, intensive care unit; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of oxygen in arterial blood; SAPS 3, simplified acute physiology score 3.

3.4. Discrimination and Accuracy of SAPS 3

The optimal cut-off SAPS 3 score was 55, 55, and 58 for the overall, non-diabetes, and diabetes cohorts, respectively. Based on these cut-off scores, sensitivity was 72.4%, 70.6%, and 66.8%; specificity was 54.5%, 56.0%, and 60.6%; positive predictive value was 50.3%, 48.8%, and 56.0%; and negative predictive value was 75.6%, 76.2%, and 70.8% for the overall, non-diabetes, and diabetes cohorts, respectively. The SAPS 3 showed unsatisfactory discrimination for all three equations (AUC = 0.69) with an insignificantly ($p = 0.193$) higher discrimination in patients with diabetes (AUC = 0.70) compared to those without diabetes (AUC = 0.68) for each equation (Table 3 and Figure 2). The Brier score was > 0.20 for all three equations in three patient cohorts, which indicated its poor accuracy in COVID-19 patients (Table 3).

Table 3. Performance of SAPS 3 standard, Central Europe, and Austrian equations in predicting in-hospital mortality in all, diabetes, and non-diabetes patients.

SAPS 3 Equations	Mortality		Discrimination	Calibration	
	Predicted Mortality Mean ± SD	SMR (95%CI)	AUROC (95%CI)	H-L X^2, *p*-Value	Brier Score
Standard equation					
All	32.47 ± 21.69	1.20 (1.16–1.24)	68.67 (67.31–70.02)	100.03, <0.001	0.22
Diabetes	34.56 ± 21.62	1.24 (1.18–1.31)	70.03 (67.53–72.53)	12.21, 0.142	0.22
No diabetes	31.63 ± 21.66	1.18 (1.13–1.22)	68.05 (66.44–69.67)	101.64, <0.001	0.22
Central Europe equation					
All	28.05 ± 21.43	1.39 (1.34–1.43)	68.67 (67.31–70.02)	120.95, <0.001	0.23
Diabetes	30.02 ± 21.56	1.43 (1.35–1.51)	70.03 (67.53–72.53)	15.08, 0.058	0.23
No diabetes	27.28 ± 21.33	1.37 (1.31–1.42)	68.05 (66.44–69.67)	119.99, <0.001	0.23
Austrian equation					
All	37.86 ± 20.56	1.03 (0.99–1.06)	68.67 (67.31–70.02)	65.10, <0.001	0.22
Diabetes	40.03 ± 20.16	1.07 (1.02–1.13)	70.03 (67.53–72.53)	9.04, 0.339	0.22
No diabetes	37.00 ± 20.66	1.01 (0.98–1.05)	68.05 (66.44–69.67)	69.55, <0.001	0.22

AUROC, area under the receiver operating characteristic curve; CI, confidence interval; H-L X^2, Hosmer–Lemeshow chi-square test; SAPS 3, simplified acute physiology score 3; SMR, standardized mortality ratio.

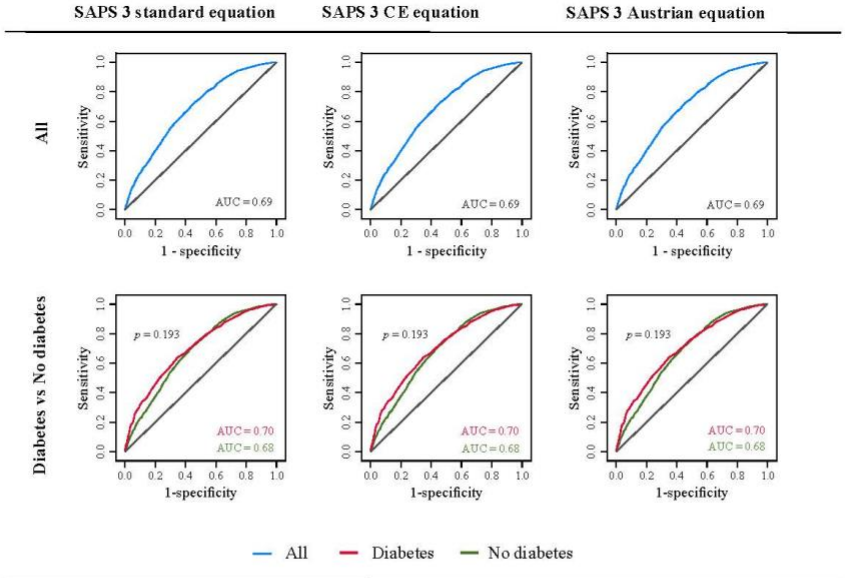

Figure 2. Receiver operating characteristics (ROC) curves for SAPS 3 standard, SAPS 3 Central Europe, and SAPS 3 Austrian equations in all, diabetes, and non-diabetes patients with COVID-19. AUC, area under the curve; CE, Central European; SAPS 3, simplified acute physiology score 3; *p*, *p*-value for DeLong test.

3.5. Calibration of SAPS 3

The SAPS 3 standard and Central European equations were miscalibrated in all three patient cohorts. Both equations underpredicted the mortality in low- and medium-risk groups and overpredicted the mortality in high-risk groups of all and non-diabetes patients. In patients with diabetes, these equations under-predicted the mortality in low- and medium-risk strata. In comparison, the Austrian recalibrated equation overpredicted the mortality in high-risk groups in the entire cohort and non-diabetes patients but had good calibration in low- and medium-risk strata. It showed reasonable calibration across all risk strata of diabetes patients as indicated by the calibration curve and H-L test ($p = 0.339$) (Table 3, Figure 3).

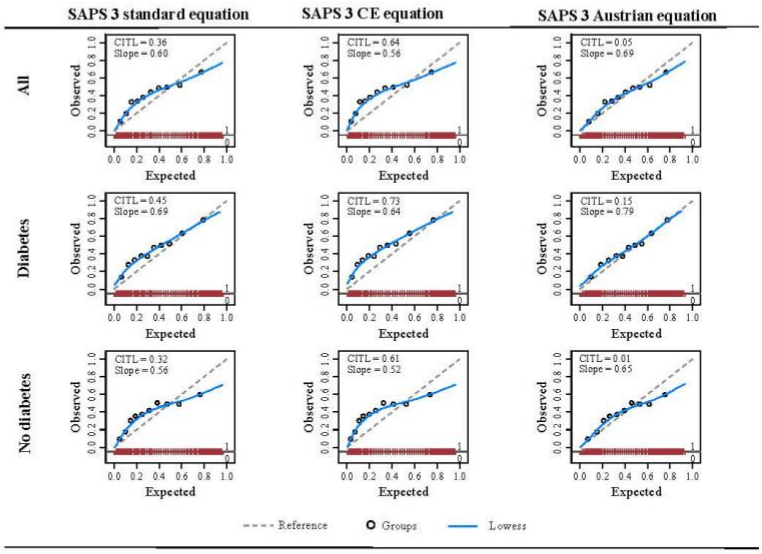

Figure 3. Calibration plots for SAPS 3 standard, SAPS 3 Central Europe, and SAPS 3 Austrian equations in all, diabetes, and non-diabetes patients with COVID-19. CE, Central European; CITL, calibration in-the-large; SAPS 3, simplified acute physiology score 3; Slope, calibration slope.

4. Discussion

This countrywide retrospective cohort analysis assessed and compared the performance of SAPS 3 for predicting the mortality in COVID-19 patients with and without diabetes using the standard and customized equations for Central Europe and Austrian COVID-19 patients. The standard and Central European equations significantly underestimated the in-hospital mortality in all three patient populations, while the Austrian equation accurately predicted in-hospital mortality in all three patient populations. The discrimination of all SAPS 3 equations was unsatisfactory in all patient cohorts and was insignificantly higher in patients with diabetes compared to those without diabetes. Likewise, the forecasting accuracy of all SAPS 3 equations was low in all cohorts. The calibration was poor for SAPS 3 standard and Central Europe equations in all three patient cohorts, and it was the worst in non-diabetes patients. The Austrian equation showed superior calibration to other SAPS 3 equations in all three populations; however, its calibration was satisfactory in diabetes patients only.

Our analysis revealed that both uncalibrated and calibrated versions of SAPS 3 equations demonstrated unsatisfactory discriminatory performance (AUC = 0.69) and accuracy (Brier score > 0.20) in patients with COVID-19. Although the performance of various

prognostication scores has been evaluated in COVID-19 patients, surprisingly, only a few studies have validated the SAPS 3 in this patient population. Compared to our study, a recent research letter reported the discrimination of SAPS 3 (AUC = 0.75) in 1464 patients admitted to ICUs in Austria. However, it remarkably underestimated the in-hospital mortality (SMR = 1.20) especially in low-risk groups, thereby questioning its clinical applicability [6]. Another research letter showed that the discrimination of the SAPS 3 regional equation was good (AUC = 0.83) with a well-concorded SMR (0.95) in Brazilian COVID-19 patients [5]. We speculate that the SAPS 3 tool has yielded different discrimination in Brazilian and Austrian patients due to differences in healthcare infrastructure and other healthcare-related factors, treatment regimens, the severity of disease, and the distribution of these risk factors in the population under study [8]. In addition, the underlying risk factors and the magnitude of their coefficients that comprise the tool are central to the discriminatory performance of a tool [9]. Furthermore, the SAPS 3 simplifies significant factors such as old age [10] and physiological disturbances into categories, which may provide inappropriate coefficients of associations for predicting in-hospital mortality in COVID-19 patients [4]. This particular issue for some SAPS 3 risk factors was highlighted by a multicenter European study [11]. Moreover, COVID-19 is more prevalent in people with multimorbidity and affects multiple body systems, and several inflammatory, coagulation, and cardiac markers have been shown to predict its severity and adverse outcomes [12]. However, the SAPS 3 score does not incorporate all these factors and markers into its equation, which might have resulted in underpredicting the mortality and henceforth its poor predictive performance in COVID-19 patients [13,14].

While validating the performance of risk tools in a specific population, satisfactory discrimination alone does not guarantee that the very tool performs well in different risk strata of patients. For this reason, achieving an optimal calibration is equally important for accurately classifying patients into risk strata and henceforth making accurate clinical decisions. Considering that COVID-19 is a debilitating infection with a high mortality rate, the accurate identification of high-risk COVID-19 patients could be vital for their clinical management and prognosis. However, in our study, the SAPS 3 standard and Central European equations were extremely miscalibrated particularly in low- and medium-risk strata of patients. These findings are not surprising, as previous validation studies also found inadequate calibration for SAPS 3 in the Austrian and Brazilian COVID-19 patients. However, in the Brazilian COVID-19 patients, the miscalibration was more obvious in high-risk groups, while similar to our findings, it was more apparent in low-risk groups in the Austrian COVID-19 patients [5,6]. The issue of miscalibration for SAPS 3 standard equations has been well documented in various patient populations [11,15,16], which indicates that this tool does not perform well in specific populations due to various patient characteristics, healthcare-related factors, variability in the coefficient of association between some risk factors and mortality, and the level of predicted outcome in the population [11]. Consequently, poor calibration of SAPS 3 compromises its clinical utility in COVID-19 patients, a fact that clinicians should be aware of.

Given the above-mentioned reasons, recalibration of the SAPS 3 has been recommended prior to applying to any patient population [5,6]. Therefore, we also adopted the recently published SAPS 3 equation for COVID-19 patients to evaluate its predictive performance in our cohort of COVID-19 patients [6]. As expected, this equation was superior to standard and Central European equations for predicting the mortality as indicated by the SMR close to 1. However, interestingly, this equation overpredicted the mortality in high-risk groups in the entire cohort and non-diabetes patients but exhibited satisfactory calibration in patients with diabetes. As mentioned earlier, the uncalibrated equations showed a similar pattern of miscalibration in the Austrian COVID-19 patients in our study and the previous study [6]. Hence, it is possible that recalibrating the equation specifically for low-risk groups might have induced the miscalibration in high-risk groups as shown in our study. The selective adequate calibration of this SAPS 3 equation in diabetes patients is a conundrum when, in fact, diabetes is not included as a risk factor in this tool. We can

only conjecture that people with diabetes are more likely to have severe COVID-19 disease, a higher burden of multimorbidity and risk factors, and pronounced physiological disturbances than their counterparts [13,15]. Perhaps that is why even uncalibrated equations showed better calibration in these patients. Nevertheless, our findings suggest that even the recalibrated equation of SAPS 3 have performed inadequately in COVID-19 patients, and therefore, this tool ought to be used with caution in this population.

As stated above, people with diabetes may experience severe COVID-19 infection, its complications, and mortality due to compromised immune and inflammatory response, advanced age, multimorbidity, and metabolic derangements [13,17,18]. Hence, we expected that the SAPS 3 will exhibit superior discriminatory performance in patients with diabetes in comparison with non-diabetes. On the contrary, the discrimination was only ~2% ($p = 0.193$) higher in patients with diabetes than without diabetes. One probable reason for the similar discrimination might be related to the inherent risk factors that are considered in the calculation of SAPS 3 score. To elaborate, the SAPS 3 is not designed for any specific disease. Rather, it is based on comprehensive patient characteristics, previous health status and therapeutic interventions, surgical status, and physiological markers, which are not specific to diabetes and hence could be altered in ICU patients with any pathophysiological condition [4]. These afore-mentioned reasons further support our findings that SAPS 3 may not be an appropriate prognostic tool for many clinical conditions including COVID-19.

This study has several limitations. First, diabetes was not classified into type 1 and type 2 diabetes because of the issue of miscoding in ICD-10 codes. Second, in-hospital mortality was defined as death occurring from any underlying causes. This could have included non-COVID-19-related deaths. Nevertheless, as this database captures data for COVID-19 patients only, the probability of including other causes of death is minimal. Third, the predictive performance of the SAPS 3 is significantly influenced by characteristics of patients, distribution of risk factors comprising the SAPS 3 score, and the healthcare system under study. Therefore, the findings of our study may not be transferable to other COVID-19 cohorts.

5. Conclusions

To conclude, SAPS 3 showed low discrimination and accuracy in Austrian COVID-19 patients, which was insignificant between diabetes and non-diabetes patients. Both uncalibrated and European calibrated equations of SAPS 3 were extremely miscalibrated especially in non-diabetes patients. We therefore recommend investigating specific determinants of SAPS 3 discrimination and calibration in COVID-19 patients. Moreover, even though the Austrian equation calibrated for COVID-19 patients demonstrated a better calibration especially in patients with diabetes, its low discrimination and forecasting power suggests that even calibrated SAPS 3 versions should be administered with caution in COVID-19 patients and revalidated locally. In addition, it would be prudent to re-evaluate its predictive performance periodically and update it as required to incorporate the impact of changes in the SARS-CoV-2 virus characteristics and treatment regimens. Furthermore, as both standard and recalibrated equations of SAPS 3 demonstrated better predictive performance in COVID-19 patients with diabetes compared to non-diabetes patients, we recommend further studies investigating this phenomenon.

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Informed Consent Statement: No consent forms were obtained from the study participants, as it was the retrospective analysis of pseudonymized data.

Data Availability Statement: The dataset used in this study is a property of the Austrian National Public Health Institute (Gesundheit Österreich GmbH). Further information regarding data access is available at: https://datenplattform-covid.goeg.at/english, accessed on 3 May 2021.

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7.4. Appendix 4

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Biomarkers Predictive for In-Hospital Mortality in Patients with Diabetes Mellitus and Prediabetes Hospitalized for COVID-19 in Austria: An Analysis of COVID-19 in Diabetes Registry

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Abstract: Background: This study assessed the predictive performance of inflammatory, hepatic, coagulation, and cardiac biomarkers in patients with prediabetes and diabetes mellitus hospitalized for COVID-19 in Austria. Methods: This was an analysis of a multicenter cohort study of 747 patients with diabetes mellitus or prediabetes hospitalized for COVID-19 in 11 hospitals in Austria. The primary outcome of this study was in-hospital mortality. The predictor variables included demographic characteristics, clinical parameters, comorbidities, use of medication, disease severity, and laboratory measurements of biomarkers. The association between biomarkers and in-hospital mortality was assessed using simple and multiple logistic regression analyses. The predictive performance of biomarkers was assessed using discrimination and calibration. Results: In our analysis, 70.8% had type 2 diabetes mellitus, 5.8% had type 1 diabetes mellitus, 14.9% had prediabetes, and 8.6% had other types of diabetes mellitus. The mean age was 70.3 ± 13.3 years, and 69.3% of patients were men.

A total of 19.0% of patients died in the hospital. In multiple logistic regression analysis, LDH, CRP, IL-6, PCT, AST-ALT ratio, NT-proBNP, and Troponin T were significantly associated with in-hospital mortality. The discrimination of NT-proBNP was 74%, and that of Troponin T was 81%. The calibration of NT-proBNP was adequate ($p = 0.302$), while it was inadequate for Troponin T ($p = 0.010$). Conclusion: Troponin T showed excellent predictive performance, while NT-proBNP showed good predictive performance for assessing in-hospital mortality in patients with diabetes mellitus hospitalized with COVID-19. Therefore, these cardiac biomarkers may be used for prognostication of COVID-19 patients.

Keywords: COVID-19; SARS-CoV-2; diabetes mellitus; biomarker

1. Introduction

In late 2019, a novel coronavirus was discovered in Wuhan, China, which was later named COVID-19. COVID-19 spread rapidly around the globe and was declared a pandemic on 11 March 2020 [1]. As of 8 April 2022, there have been 494,587,638 confirmed cases of COVID-19 and 6,170,283 deaths worldwide [1]. At this time in Austria, there have been 3,998,130 cases of COVID-19 and 15,982 deaths [1].

Studies from China, Austria, and other European countries investigating the association between diabetes mellitus and COVID-19 have found that diabetes mellitus adversely affects the prognosis of COVID-19 via various mechanisms. Hyperglycemia increases inflammation by producing oxidative stress and consequently increases the risk of cytokine storm [2]. Hyperglycemia also supports viral proliferation via increased viral replication in monocytes [2]. The antiviral immune and inflammatory responses in infected patients with diabetes mellitus can also change insulin sensitivity by impairing glucose metabolism [2]. It has been reported that patients with diabetes mellitus do not contract COVID-19 infection more frequently than people without diabetes; however, a higher prevalence, a severe and prolonged course of the disease, and increased mortality have been shown in people with diabetes [3]. In this regard, studies have shown that diabetes is present in 17–33% of hospitalized COVID-19 patients [4–6]. Other studies have reported that patients with diabetes are 15% more likely to be admitted to critical care units [7], 2.6 times more likely to develop severe infection, and 2 times more likely to die compared with those without diabetes [8]. The above-described mechanisms of oxidative stress and pathophysiological cytokine production may also induce endothelial damage, which results in thromboembolic events and organ damage and therefore leads to higher complication rates [2].

As COVID-19 is considered a multisystem disease, studies have reported that various inflammatory, hepatic, coagulation, and cardiac biomarkers are correlated with COVID-19 disease severity and mortality. Specifically, elevated levels of CRP, procalcitonin, and interleukin-6 have been associated with increased mortality. Moreover, cardiac injury has been reported in 20% of patients with COVID-19; therefore, cardiac markers such as NT-proBNP and Troponin have also been found significantly elevated in those who died or had severe disease [9–11]. Furthermore, in patients with diabetes mellitus, D-dimer has been reported as a significant predictor of COVID-19 mortality [12], while CRP has been associated with a severe course of COVID-19 in patients [13]. However, evidence regarding the role of biomarkers in COVID-19 patients with diabetes mellitus is still scarce. Therefore, we assessed the predictive performance of various inflammatory, hepatic, coagulation, and cardiac biomarkers in patients with prediabetes and diabetes mellitus hospitalized for COVID-19.

2. Materials and Methods

2.1. Study Design

We performed a retrospective analysis of COVID-19 patients who are enrolled in the COVID-19 diabetes registry. The COVID-19 diabetes registry is an ongoing multicenter

cohort study of diabetes mellitus patients who are hospitalized with confirmed SARS-CoV-2 infection in 11 participating hospitals in Austria. This study was initiated on 15 April 2020 and is sponsored by the Austrian Diabetes Association. For this analysis, data collected until 30 April 2021 were considered. The methodological details of this study are described in the study published by Sourij et al. [14].

2.2. Study Population and Inclusion Criteria

Both men and women aged ≥18 years with a positive throat swab for SARS-CoV-2 and a confirmed diagnosis of diabetes mellitus or prediabetes were enrolled in this analysis. Types of diabetes mellitus include type 1 diabetes mellitus, type 2 diabetes mellitus, and other types of diabetes mellitus. Diabetes mellitus was diagnosed according to the Austrian Diabetes Association criteria and prediabetes was defined as glycated haemoglobin (HbA1c) of 5.7–6.4% (39–46 mmol/mol), which was measured if glucose levels were elevated in patients without a known diagnosis of diabetes mellitus [15]. In the current data analysis, only patients hospitalized for COVID-19 were included.

2.3. Data Collection

Designated study coordinators and clinicians at each participating hospital collected pseudonymized data from eligible patients using an electronic case report form (CRF). Clinical variables were collected from the medical files of patients and the values of biomarkers and other laboratory measures were collected from the local clinical laboratory of each participating hospital.

2.4. Study Variables

2.4.1. Outcome

The primary outcome of this study is in-hospital mortality, which is defined as death from the date of admission for COVID-19 to the date of discharge from the hospital.

2.4.2. Predictors

The predictor variables were collected at the time of admission and comprised data related to demographic characteristics, clinical parameters, comorbidities, use of medication, course of the disease, and laboratory measurements.

Demographic characteristics included age and gender. Clinical data included weight, height, oxygen saturation, systolic and diastolic blood pressure, pulse, smoking status, and classification and duration of diabetes mellitus. Data related to the course of the disease included length of stay, ICU requirement, assisted ventilation, and specific COVID-19 therapy.

Comorbidities included heart failure, coronary heart disease (CHD), hypertension, central arterial occlusion disease, peripheral arterial occlusion disease, stroke, myocardial infarction, chronic kidney disease (CKD), autoimmune diseases, tumor diseases, chronic obstructive pulmonary disease (COPD), bronchial asthma, interstitial lung diseases, transplantations, and various liver diseases, such as non-alcoholic fatty liver disease (NAFLD) (biopsy-confirmed and patient-reported), congenital diseases (e.g., Mb. Wilson), viral hepatitis, cancer, and alcohol abuse.

The following oral antidiabetic drugs were charted: metformin, sulfonylureas, dipeptidyl-peptidase-4 inhibitors, sodium-dependent glucose co-transporter-2 inhibitors, and glucagon-like peptide receptor agonists. Insulin therapy was distinguished as premixed insulin, basal insulin, bolus insulin, and/or insulin pump therapy. Concomitant medications included blood-pressure-regulating drugs (angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, calcium antagonists, central antihypertensives, thiazides, loop diuretics, mineral corticoid receptor blockers, and sacubitril), immune-regulating drugs (glucocorticoids and other immunosuppressants), anticoagulants, and pain medications (ibuprofen). Specific COVID-19 therapy was recorded as the use of antiviral, antibiotic, and antifungal drugs, corticosteroids, and anticoagulants.

Laboratory measurements included random glucose, HbA1c, plasma lipoproteins (total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and triglycerides), hematological markers (leukocytes, hemoglobin, platelets), renal markers (estimated glomerular filtration rate (eGFR)), hemolysis parameter (lactate dehydrogenase (LDH)), liver enzymes (glutamate oxaloacetate transaminase/aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/alanine aminotransferase (GPT/ALT), and gamma-glutamyl transferase (GGT)), inflammation markers (C-reactive protein (CRP), ferritin, procalcitonin (PCT), and interleukin-6 (IL-6)), coagulation markers (fibrinogen and D-dimer), and cardiac markers (N-terminal pro-B-type natriuretic peptide (NT-proBNP), and Troponin T).

2.5. Ethical Considerations

The study was approved on 15 April 2020 by the Ethics Committee of the Medical University of Graz, Graz, Austria (EK 32-355 ex 19/20) with subsequent amendments for countrywide data collection. Written informed consent was obtained from living patients to participate in the study, if possible. Patients who were unable to provide informed consent before their hospital discharge were contacted later to agree on the use of their clinical data. For patients who died before they could provide consent or where it was not possible to obtain consent, the Ethics Committee waived the need for informed consent. All data were anonymized prior to the analysis.

2.6. Statistical Analysis

For this study, data were extracted in Microsoft Excel format and analyzed in Stata version 17.0 and R studio version 2022.02.0. Categorical variables are described as frequencies with corresponding percentages (%). Quantitative variables are described as mean ± standard deviation (SD) or median with interquartile range [IQR] if the normal distribution was violated. Normality of quantitative variables was assessed using the Shapiro–Wilk test. Categorical variables were compared with in-hospital mortality using the Chi-square test. Quantitative variables were compared with in-hospital mortality using the unpaired t-test or their non-parametric equivalent Wilcoxon rank-sum test.

As all biomarkers were positively skewed, they were therefore log-transformed before further analysis. The correlation between each log-transformed biomarker was assessed using the Pearson correlation method and displayed as a scatterplot with the corresponding correlation coefficient and the *p*-value. The association between transformed biomarkers and in-hospital mortality was then assessed using simple and multiple logistic regression analyses. In simple logistic regression, the association of each biomarker was assessed individually with in-hospital mortality. In multiple logistic regression, the association of each biomarker with in-hospital mortality was adjusted for age, sex, and type of diabetes mellitus. The predictive performance of biomarkers was assessed using discrimination and calibration. Discrimination was measured in terms of concordance statistics (C-statistic) and displayed as an area under the receiver operator characteristic curve (AUC) plot. Calibration was measured by the Hosmer–Lemeshow goodness-of-fit test.

3. Results

3.1. Characteristics of Study Participants, Overall and by In-Hospital Mortality

The distribution of characteristics, comorbidities, and biomarkers of patients with diabetes mellitus hospitalized for COVID-19 are shown in Table 1. In total, 747 people were included in the analysis. The mean age was 70.3 ± 13.3 years. There were 518 (69.3%) males and 229 (30.7%) females enrolled in the analysis. The mean body mass index (BMI) was 29.0 ± 5.9 kg/m^2. Most patients had type 2 diabetes mellitus (70.8%), followed by prediabetes (14.9%), other types of diabetes mellitus (8.6%), and type 1 diabetes mellitus (5.8%). Common comorbidities were hypertension (68.0%), coronary heart disease (26.5%), chronic kidney disease (21.4%), respiratory disease (19.7%), and peripheral artery disease (13.9%).

Table 1. Comparison of characteristics, comorbidities, and biomarkers with in-hospital mortality in patients with prediabetes and diabetes mellitus hospitalized with COVID-19.

Variables	*n*	All	In-Hospital Mortality		*p*-Value
			Yes	No	
All, *n* (%)	747	–	142 (19.0)	605 (81.0)	–
Characteristics					
Age—years, mean ± SD	717	70.3 ±13.3	78.63 ±10.0	68.3 ±13.2	<0.001
Sex, *n* (%)	747				
Male		518 (69.3)	95 (66.9)	423 (69.9)	0.483
Female		229 (30.7)	47 (33.1)	182 (30.1)	
Smoking status, *n* (%)	747				
Non-smoker		372 (49.8)	70 (49.3)	302 (49.9)	0.399
Former smoker		97 (13.0)	24 (16.9)	73 (12.1)	
Current smoker		23 (3.1)	5 (3.5)	18 (3.0)	
Unknown		255 (34.1)	43 (30.3)	212 (35.0)	
Body mass index—kg/m^2, mean ± SD	390	29.0 ±5.9	29.52 ±6.7	28.9 ±5.7	0.439
Type of diabetes mellitus, *n* (%)	747				
Prediabetes		111 (14.9)	12 (8.5)	99 (16.4)	0.010
Type 1 diabetes mellitus		43 (5.8)	5 (3.5)	38 (6.3)	
Type 2 diabetes mellitus		529 (70.8)	117 (82.4)	412 (68.1)	
Other diabetes mellitus		64 (8.6)	8 (5.6)	56 (9.3)	
Comorbidities					
Hypertension, *n* (%)	747	508 (68.0)	112 (78.9)	396 (65.5)	0.002
Coronary heart disease, *n* (%)	747	198 (26.5)	52 (36.6)	146 (24.1)	0.002
Myocardial infarction, *n* (%)	747	90 (12.1)	25 (17.6)	65 (10.7)	0.024
Heart failure, *n* (%)	747	91 (12.2)	38 (26.8)	53 (8.8)	<0.001
Peripheral artery disease, *n* (%)	747	104 (13.9)	38 (26.8)	66 (10.9)	<0.001
Stroke, *n* (%)	747	57 (7.6)	16 (11.3)	41 (6.8)	0.070
Chronic kidney disease, *n* (%)	747	160 (21.4)	52 (36.6)	108 (17.9)	<0.001
Cancer, *n* (%)	747	90 (12.1)	28 (19.7)	62 (10.3)	0.002
Respiratory disease, *n* (%)	747	147 (19.7)	35 (24.7)	112 (18.5)	0.098
Liver disease, *n* (%)	747	57 (7.6)	14 (9.9)	43 (7.1)	0.266
Inflammatory biomarkers					
LDH—U/L, median [IQR]	681	288.0 [160.0]	311.5 [165.0]	281.0 [159.0]	0.147
CRP—mg/dL, median [IQR]	711	12.1 [43.5]	20.4 [66.9]	10.7 [34.2]	<0.001
IL6—pg/mL, median [IQR]	489	41.8 [56.8]	67.7 [80.7]	38.5 [49.3]	<0.001
PCT—ng/mL, median [IQR]	503	0.1 [0.1]	0.2 [0.4]	0.1 [0.1]	<0.001
Ferritin—ng/mL, median [IQR]	555	568.0 [938.0]	562.0 [864.0]	570.0 [944.0]	0.559
Hepatic biomarkers					
AST—U/L, median [IQR]	565	38.0 [29.0]	42.0 [34.5]	36.0 [28.0]	0.027
ALT—U/L, median [IQR]	578	29.0 [25.0]	27.0 [20.0]	29.0 [27.0]	0.037
AST–ALT ratio	564	1.33 [0.8]	1.67 [1.0]	1.28 [0.7]	<0.001
Coagulation biomarkers					
D-dimer—mcg/mL, median [IQR]	140	0.99 [0.97]	1.28 [4.08]	0.90 [0.98]	0.016
Cardiac biomarkers					
NT-proBNP—pg/mL, median [IQR]	296	418.5 [1464.0]	1333.5 [5003.5]	297.0 [730.0]	<0.001
Troponin T—pg/mL, median [IQR]	242	20.0 [31.0]	43.0 [44.0]	16.0 [22.0]	<0.001

LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine transaminase; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

A total of 142 (19.0%) patients died in the hospital during the study period. Patients who died (78.6 ± 10.0 years) were significantly ($p < 0.001$) older compared to the survivors (68.3 ± 13.2 years). In addition, the prevalence of hypertension was significantly ($p = 0.002$) higher in patients who died (78.9%) compared to the survivors (65.5%). Similar results were noted for coronary heart disease (36.6% vs. 24.1%, $p = 0.002$), myocardial infarction (17.6% vs. 10.7%, $p = 0.024$), heart failure (26.8% vs. 8.8%, $p < 0.001$), peripheral artery disease (26.8% vs. 10.9%, $p < 0.001$), chronic kidney disease (36.6% vs 17.9%, $p < 0.001$), and cancer (19.7% vs. 10.3%, $p = 0.002$).

3.2. Biomarker of COVID-19 Mortality

The median [IQR] values of biomarkers and their comparison by in-hospital mortality status are shown in Table 1 and Figure 1. For inflammatory markers, the median value of LDH was 288.0 U/L [160.0], CRP was 12.1 mg/dL [43.4], IL-6 was 41.8 pg/mL [56.8], PCT was 0.1 ng/mL [0.1], and ferritin was 568.0 ng/mL [938.0]. The median AST–ALT ratio was 1.3 [0.8], and coagulation marker D-dimer was 1.0 mcg/mL [1.0]. For cardiac markers, the median level of NT-proBNP was 418.5 pg/mL [1464.0], and Troponin T was 20.0 [31.0] pg/mL.

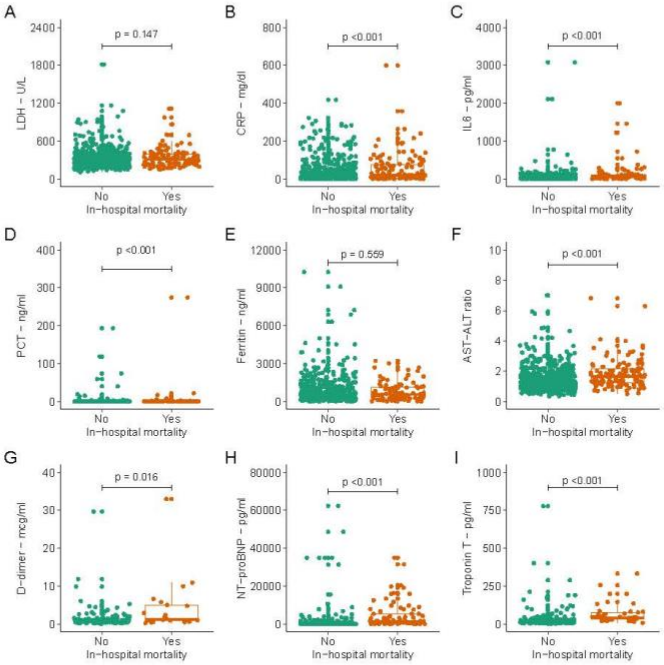

Figure 1. Distribution of biomarkers by in-hospital mortality. Each subplot (**A**–**I**) compares the distribution of biomarker between patients who died in the hospital and those who survived. Y-axis represents the range of values for each biomarker and X-axis represents patients who died versus those who were alive. LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NT-proBNP: N-terminal pro-brain natriuretic peptide. *p* = Wilcoxon rank-sum test *p*-value.

Comparison of biomarkers with in-hospital mortality shows that the median levels of CRP (20.4 [66.9] vs. 10.7 [34.2], $p < 0.001$), IL-6 (67.7 [80.7] vs. 38.5 [49.3], $p < 0.001$), and PCT (0.2 [0.4] vs. 0.1 [0.1], $p < 0.001$) were significantly higher in patients who died in the hospital compared to those who survived. However, the median levels of LDH (311.5 [165.0] vs. 281.0 [159.0], $p = 0.147$) and ferritin (562.0 [864.0] vs. 570.0 [944.0], $p = 0.559$) did not differ significantly between non-survivors and survivors. For hepatic markers, the median levels of AST (42.0 [34.5] vs. 36.0 [28.0], $p = 0.027$), ALT (27.0 [20.0] vs. 29.0 [27.0], $p = 0.037$), and AST–ALT ratio (1.7 [1.0] vs. 1.3 [0.7], $p < 0.001$) were significantly higher in non-survivors versus survivors. The median level of coagulation marker D-dimer (1.3 [4.1] vs. 0.9 [1.0], $p < 0.001$) was significantly higher in people who died compared to those who survived. Similar results were observed for the cardiac markers NT-proBNP (1333.5 [5003.5] vs. 297.0 [730.0], $p < 0.001$) and Troponin T (43.0 [44.0] vs. 16.0 [22.0], $p < 0.001$) (Figure 1).

3.3. Correlation between Biomarkers

Figure 2 shows the Pearson correlation analyses between biomarkers. IL-6 had a moderate positive correlation with all other biomarkers. NT-proBNP had a strong positive correlation with Troponin T (r = 0.70, $p < 0.001$), a weak positive correlation with IL-6 (r = 0.22, $p = < 0.001$), and a moderate positive correlation with PCT (r = 0.30, $p < 0.001$), AST–ALT ratio (r = 0.37, $p < 0.001$), and D-dimer (r = 0.38, $p < 0.001$). Ferritin was moderately positively correlated with LDH (r = 0.46, $p < 0.001$) and CRP (r = 0.32, $p < 0.001$). There was a moderate positive correlation between PCT, NT-proBNP, Troponin T, and IL6. LDH was moderately positively correlated with every biomarker except NT-proBNP and Troponin T. The AST–ALT ratio was moderately positively correlated with other biomarkers, except ferritin and PCT. D-Dimer was moderately positively correlated with all biomarkers except PCT and ferritin.

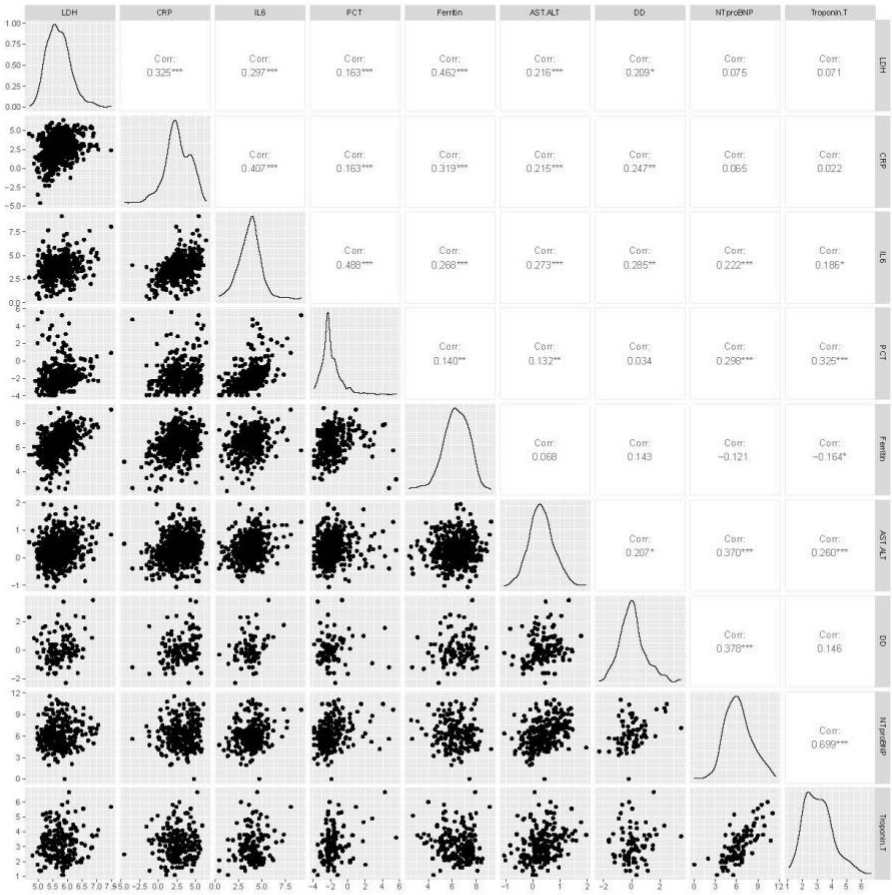

Figure 2. Pearson correlation plots of log-transformed biomarkers. X and Y labels correspond to log-transformed values of biomarkers. Corr: corresponds to Pearson correlation coefficient. LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NT-proBNP: N-terminal pro-brain natriuretic peptide. * The correlation is significant at < 0.05. ** The correlation is significant at < 0.01. *** The correlation is significant at < 0.001.

3.4. Association of Biomarkers with In-Hospital Mortality

In simple logistic regression analysis, CRP, IL-6, PCT, D-dimer, AST–ALT ratio, NT-proBNP, and Troponin T were significantly associated with in-hospital mortality (Table 2). In multiple logistic regression analysis, which was adjusted for age, sex, and type of diabetes mellitus, CRP, IL-6, PCT, AST–ALT ratio, NT-proBNP, and Troponin T remained significantly associated with in-hospital mortality. While LDH became a significant predictor of mortality in the multiple logistic regression analysis, D-dimer did not retain significance in the multiple logistic regression analysis. Ferritin was not significantly associated with in-hospital mortality in either simple or multiple logistic regression analysis.

Table 2. Logistic regression analysis of biomarkers with in-hospital mortality.

Biomarkers	Simple Logistic Regression			Multiple Logistic Regression		
	OR	95%CI	*p*-Value	AOR	95%CI	*p*-Value
Inflammatory biomarkers						
LDH—U/L	1.4	0.88–2.25	0.158	2.03	1.21–3.42	0.008
CRP—mg/dL	1.3	1.15–1.47	<0.001	1.33	1.16–1.52	<0.001
IL6—pg/mL	1.66	1.34–2.06	<0.001	1.6	1.27–2.01	<0.001
PCT—ng/mL	1.31	1.13–1.51	<0.001	1.25	1.06–1.48	0.007
Ferritin—ng/mL	0.9	0.74–1.10	0.3	1.07	0.86–1.35	0.541
Coagulation biomarkers						
D-dimer—mcg/mL	1.93	1.22–3.03	0.005	1.66	0.97–2.82	0.063
Hepatic biomarkers						
AST–ALT ratio	3	1.97–4.56	<0.001	1.89	1.19–3.01	0.007
Cardiac biomarkers						
NT-proBNP—pg/mL	1.59	1.35–1.86	<0.001	1.5	1.24–1.80	<0.001
Troponin T—pg/mL	2.78	1.90–4.07	<0.001	2.2	1.44–3.35	<0.001

LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NT-proBNP: N-terminal pro-brain natriuretic peptide. Multiple logistic regression model was adjusted for age, sex, and type of diabetes mellitus.

3.5. Predictive Performance of Biomarkers

NT-proBNP showed good discrimination with an AUC of 0.74, and Troponin T showed excellent discrimination with an AUC of 0.81 (Figure 3), while all other investigated biomarkers did not show good discrimination. NT-proBNP showed good calibration too (H-L statistics = 9.50, $p = 0.302$), whereas Troponin T showed poor calibration (H-L statistics = 20.03, $p = 0.010$) (Table 3).

Table 3. Hosmer–Lemeshow goodness-of-fit test of biomarkers.

Biomarkers	Hosmer–Lemeshow Test	
	Statistics	*p*-Value
Inflammatory biomarkers		
LDH—U/L	3.22	0.920
CRP—mg/dL	5.82	0.667
IL6—pg/mL	3.81	0.874
PCT—ng/mL	6.63	0.577
Ferritin—ng/mL	6.42	0.600
Coagulation biomarkers		
D-dimer—mcg/mL	6.19	0.626
Hepatic biomarkers		
AST–ALT ratio	7.96	0.437
Cardiac biomarkers		
NT-proBNP—pg/mL	9.50	0.302
Troponin T—pg/mL	20.03	0.010

LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NT-proBNP: N-terminal pro-brain natriuretic peptide.

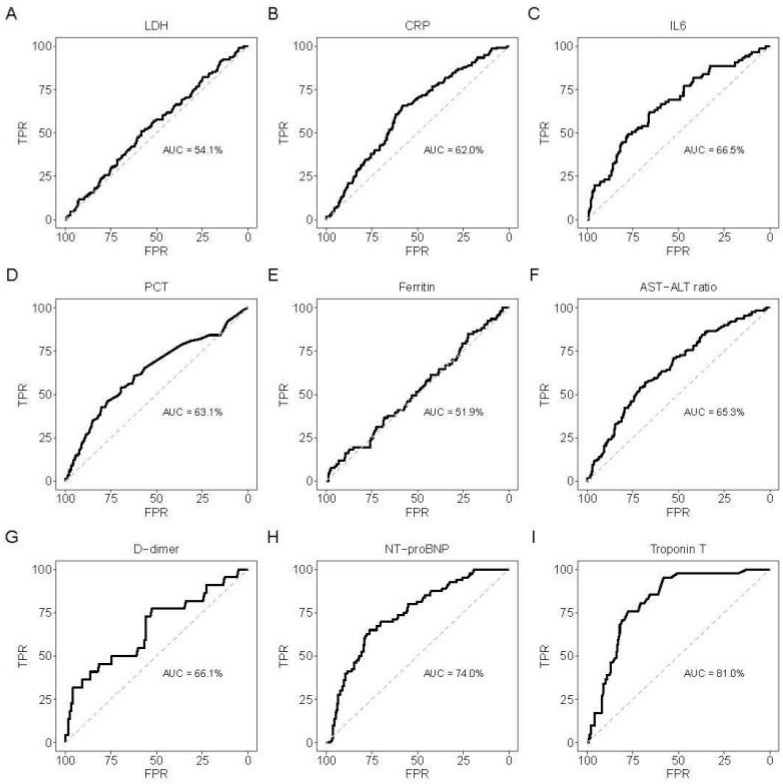

Figure 3. ROC curve plots of biomarkers with in-hospital mortality. In each subplot (**A**–**I**), grey diagonal line shows no predictive value and black line shows the actual predictive curve for each biomarker. Y axis represents TPR while X-axis represents FPR. AUC: area under the curve; TPR: true positive rate; FPR: false positive rate; LDH: lactate dehydrogenase; CRP: C-reactive protein; IL6: interleukin 6; PCT: procalcitonin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NT-proBNP: N-terminal pro-brain natriuretic peptide.

4. Discussion

In this multicenter retrospective cohort analysis of people with prediabetes and diabetes mellitus hospitalized with COVID-19, we assessed the performance of various inflammation, hepatic, coagulation, and cardiac biomarkers for predicting in-hospital mortality. Of the total number of hospitalized individuals, 70.8% had type 2 diabetes mellitus, 5.8% had type 1 diabetes mellitus, 14.9% had prediabetes, and 8.6% had other types of diabetes mellitus, and a total of 19.0% of patients died in the hospital. Of the studied biomarkers, the cardiac biomarkers NT-proBNP (74.0%) and Troponin T (81.0%) showed satisfactory predictive performance for in-hospital mortality, while none of the inflammatory, hepatic, or coagulation markers demonstrated a satisfactory predictive performance in our cohort.

In this study, the in-hospital mortality was 19.0% in patients with prediabetes and diabetes mellitus. Compared to our findings, Hammad et al. reported a higher in-hospital mortality rate of 38.2% in patients with diabetes mellitus, while Acharya et al. reported a similar mortality rate of 20.0% in patients with diabetes mellitus compared to 7.4% in the whole population [16,17]. In contrast with other studies, the in-hospital mortality was not significantly different between males and females in our analysis; however, the populations

being hospitalized are not directly comparable between countries with different health care systems [17,18].

Our analysis showed significantly higher NT-proBNP and Troponin T levels in patients who died in the hospital compared to those who survived. In addition, the elevated levels of these cardiac biomarkers were significantly associated with increased in-hospital mortality. These associations remained significant even after excluding patients with a history of heart failure and adjusting for other comorbidities, which further confirms the independent association of cardiac biomarkers with mortality in our cohort. Only a few other studies have investigated the role of biomarkers with adverse outcomes in patients with diabetes mellitus following a COVID-19 infection. Reviews by Ceriello et al., Lippi et al., Bavishi et al., and Lavie et al. highlighted the importance of measuring NT-proBNP and Troponin to aid in the diagnosis and prognostication of COVID-19 in patients with and without diabetes mellitus because of the high risk of cardiac injury in this population [10,11,19,20]. A recent meta-analysis also showed increased levels of Troponin in people with severe COVID-19 infection [21]. Another study from China comprising 28 patients with diabetes mellitus and COVID-19 also reported that NT-proBNP was significantly increased in ICU patients compared to non-ICU patients [22]. Studies assessing the predictive performance of cardiac biomarkers in patients with diabetes mellitus are scarce; however, Cunningham et al. showed the good predictive performance of NT-proBNP (area under the ROC curve: 0.75) in patients with COVID-19 [23]. Various pathophysiological mechanisms have been identified to alter cardiac markers in COVID-19. It is postulated that viral illnesses such as COVID-19 can damage myocardial cells, especially in patients with pre-existing cardiovascular diseases, through mechanisms such as systemic inflammatory responses, direct damage by the virus, and hypoxia [24]. Hypoxia induces pulmonary hypertension, which subsequently increases ventricular wall stress and thus releases NT-proBNP [25].

Although most inflammatory biomarkers (CRP, LDH, IL6, PCT, and ferritin) were significantly associated with in-hospital mortality, none of these biomarkers demonstrated satisfactory predictive performance in our study. Jayanthy and colleagues evaluated the predictive performance of CRP in patients with COVID-19 and a subgroup of diabetes mellitus patients and demonstrated a discrimination of 0.72 in this subgroup [26]. In another study conducted by Wang and colleagues comprising patients with diabetes mellitus, LDH, PCT, CRP, ferritin, and IL-6 were significantly increased in ICU patients compared to non-ICU patients [22]. However, they did not evaluate the predictive performance of these biomarkers. Hammad and colleagues showed abnormal albeit insignificant LDH and CRP levels in their patients ($n = 118$) with diabetes mellitus [17], while Bhatti and colleagues, in their analysis of 103 patients with diabetes mellitus and prediabetes with COVID-19, showed significantly higher levels of ferritin and CRP in patients who needed ICU care [18]. The discrepancies in the findings regarding the predictive role of inflammatory markers in patients with diabetes warrant further studies in larger patient cohorts.

The coagulation biomarker d-dimer was significantly associated with in-hospital mortality; however, its discrimination was unsatisfactory (0.66) in our cohort. In comparison, Miri and colleagues reported an AUC of 0.75 for d-dimer in COVID-19 patients with diabetes [10]. Furthermore, the study by Wang and colleagues showed D-dimer to be significantly increased in ICU patients compared to non-ICU patients with diabetes mellitus. Likewise, Bhatti and colleagues showed significantly higher levels of d-dimer in patients who needed ICU care. However, these studies did not explore the predictive role of d-dimer [18,22]. The association between elevated d-dimer and in-hospital mortality in patients with diabetes mellitus could be explained by hyperglycemia-related alterations in the thrombosis cascade [27].

For hepatic biomarkers, we analyzed only the AST–ALT ratio and found it to be significantly associated with in-hospital mortality, albeit with inadequate predictive performance. Hammad and colleagues also showed increased ALT to be a significant positive predictor of mortality in patients with diabetes mellitus and COVID-19 [17]. Elevated liver enzymes indicate liver damage, which could result from immune-related injury or direct liver cy-

totoxicity. However, liver-damaging medications, such as some antivirals and antibiotics, which are used in the treatment of COVID-19, could also elevate liver enzymes [28].

Our study is not without limitations. One limitation is the unavailability of comparison data for patients without diabetes mellitus who were hospitalized with COVID-19. Another limitation is the limited number of people with prediabetes in our study. For this reason, we could not perform a comparative analysis between patients with prediabetes and diabetes mellitus. As often encountered in routinely collected health data, the classification of diabetes mellitus into subtypes may not be accurate. In addition, due to the pragmatic design of this study, laboratory data were not available for all patients. Furthermore, biomarkers were measured only at the time of admission, which may not fully reflect the clinical course and prognosis of COVID-19.

The major strengths of the study are its adequate sample size, the longitudinal study design, and the inclusion of multiple hospitals across Austria. The adjustment for known confounding factors to determine the independent association of biomarkers with in-hospital mortality is another strength of our study.

5. Conclusions

In conclusion, the cardiac biomarkers Troponin T and NT-proBNP demonstrated satisfactory predictive performance in people with prediabetes and diabetes in this study. As these biomarkers can be routinely measured in the clinical setting, they could therefore be used as prognostic biomarkers for COVID-19 patients. However, we recommend more studies investigating the prognostic role of these biomarkers in larger diabetes cohorts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v14061285/s1.

Author Contributions: H.S. (Harald Sourij) and F.A. (Faisal Aziz) conceived the idea of this study. F.A. (Faisal Aziz) and H.S. (Hannah Stöcher) drafted the manuscript. F.A. (Faisal Aziz) performed all statistical analyses. A.B., C.C., M.C., P.F., M.K., A.K.-W., C.K., O.M. (Oliver Malle), F.A. (Felix Aberer), E.P., S.P., C.R., C.S. (Caren Sourij), L.S., H.S. (Harald Stingl), T.S., N.T., M.W., P.W., A.Z., O.M. (Othmar Moser), C.S. (Christian Schelkshorn) and S.K. critically revised and improved the manuscript. H.S. (Harald Sourij) and F.A. (Faisal Aziz) are guarantors of the integrity of data. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review from the Ethics Committee of the Medical University of Graz, Austria (EK 32–355 ex 19/20).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study, if possible. Patients who were unable to provide informed consent before their hospital release were contacted later to agree on the use of their clinical data. For patients who died before they could provide consent or where it was not possible to obtain consent, the Ethics Committee waived the need for informed consent.

Data Availability Statement: The data used in this study are owned by the Austrian Diabetes Association and can be provided upon request.

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Conflicts of Interest: The authors declare no conflict of interest related to this study.

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7.5. Appendix 5

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ORIGINAL ARTICLE

WILEY

Humoral immune response to COVID-19 vaccination in diabetes is age-dependent but independent of type of diabetes and glycaemic control: The prospective COVAC-DM cohort study

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Abstract

Aims: To investigate the seroconversion following first and second COVID-19 vaccination in people with type 1 and type 2 diabetes in relation to glycaemic control prior to vaccination and to analyse the response in comparison to individuals without diabetes.

Materials and methods: This prospective, multicentre cohort study analysed people with type 1 and type 2 diabetes and a glycated haemoglobin level ≤58 mmol/mol (7.5%) or >58 mmol/mol (7.5%), respectively, and healthy controls. Roche's Elecsys anti-SARS-CoV-2 S immunoassay targeting the receptor-binding domain was used to quantify anti-spike protein antibodies 7 to 14 days after the first and 14 to 21 days after the second vaccination.

Results: A total of 86 healthy controls were enrolled in the study, as well as 161 participants with diabetes, of whom 150 (75 with type 1 diabetes and 75 with type 2 diabetes) were eligible for the analysis. After the first vaccination, only 52.7% of participants in the type 1 diabetes group and 48.0% of those in the type 2 diabetes

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group showed antibody levels above the cut-off for positivity. Antibody levels after the second vaccination were similar in participants with type 1 diabetes, participants with type 2 diabetes and healthy controls after adjusting for age, sex and multiple testing ($P > 0.05$). Age ($r = -0.45$, $P < 0.001$) and glomerular filtration rate ($r = 0.28$, $P = 0.001$) were significantly associated with antibody response.

Conclusions: Anti-SARS-CoV-2 S receptor-binding domain antibody levels after the second vaccination were comparable in healthy controls and in participants with type 1 and type 2 diabetes, irrespective of glycaemic control. Age and renal function correlated significantly with the extent of antibody levels.

KEYWORDS
COVID-19, observational study, type 1 diabetes, type 2 diabetes

1 | INTRODUCTION

After the first occurrence of the SARS-CoV-2 virus causing the coronavirus disease (COVID-19) in China in December 2019, the virus has rapidly spread globally, leading to the declaration of a COVID-19 pandemic in March 2020 by the World Health Organization (WHO).[1] Reports from China,[2,3] Europe[4] and the United States[5] demonstrated that the prevalence of diabetes is as high as 20% in people hospitalized for COVID-19. Moreover, diabetes is frequent in people experiencing a severe or fatal disease course of COVID-19,[6] with an in-hospital mortality reported to be as high as 25% in people with diabetes mellitus.[7]

People with diabetes are therefore usually considered a high-risk population for experiencing adverse COVID-19 outcomes and, consequently, COVID-19 vaccination is highly recommended in this population, leading to prioritization in current vaccination strategies of most countries.[8] Given that a compromised immune response to SARS-CoV-2 has been discussed as a possible reason for the increased risk of severe COVID-19 in people with diabetes, there also remains the question of whether people with diabetes have a reduced immune response following SARS-CoV-2 vaccinations. While most studies on hepatitis B vaccination have demonstrated reduced immunogenicity in people with diabetes,[9] data on other vaccinations, including those against influenza, were mostly inconclusive.[9]

While phase III studies on both mRNA- and adenovirus-based COVID-19 vaccines have included people with diabetes and the efficacy rates at least for mRNA vaccines in people with diabetes appear to be similar to those among their counterparts without diabetes,[10-14] data on the characteristics of the included people with diabetes are sparse. Recently, a study suggested that lower antibody levels are present in response to COVID-19 vaccination in people with diabetes. However, the number of people with diabetes included was limited and there was no differentiation between type 1 and type 2 diabetes nor details of the potential impact of glycaemic control prior to receiving the vaccine.[15]

The aim of this study, therefore, was to investigate the humoral immune response and side effects related to COVID-19 vaccines in people with type 1 and type 2 diabetes in order to elucidate the impacts of type of diabetes and glycaemic control on antibody response following COVID-19 vaccinations. Moreover, we aimed to compare antibody levels to the receptor-binding domain (RBD) of the SARS-CoV-2 S protein after COVID-19 vaccination in people with diabetes to healthy, non-diabetes controls.

2 | MATERIALS AND METHODS

The "Immune response to Covid-19 vaccination in people with Diabetes Mellitus – COVAC-DM" study was a prospective, multicentre, real-world, cohort study including 161 individuals with diabetes mellitus at two centres in Austria (Medical University of Graz and Medical University of Innsbruck) and one centre in Germany (University of Bayreuth). We included adults with type 1 or type 2 diabetes, aged 18 to 80 years, who were diagnosed with diabetes prior to receiving a COVID-19 vaccine and who were willing to give written informed consent. The main exclusion criteria were: active malignancy (excluding intraepithelial neoplasia of the prostate gland and the gastrointestinal tract and basalioma); pregnancy; acute inflammatory disease; immunosuppressant therapy; alcohol abuse (more than 15 standard drinks a week); or any contraindication to the vaccine as well as a previous episode of COVID-19.

People with established type 1 or type 2 diabetes and planned COVID-19 vaccination were recruited from outpatient clinics at the participating sites, from the Graz Diabetes Registry for Biomarker Research, and through advertisements in local newspapers.

Participants were then classified according to their glycated haemoglobin (HbA1c) and type of diabetes into one of the four predefined groups: type 1 diabetes with an HbA1c level ≤58 mmol/mol (≤7.5%), type 1 diabetes with an HbA1c level >58 mmol/mol

(>7.5%), type 2 diabetes with an HbA1c level ≤58 mmol/mol (≤7.5%) and type 2 diabetes with an HbA1c level >58 mmol/mol (>7.5%).

All participants were asked to attend on-site visits 60 to 2 days prior to their first vaccination, 7 to 14 days after their first vaccination and 14 to 21 days after their second vaccination. A physical examination was performed and blood samples were taken. Data on medical history and medication were collected at baseline and information about side effects from vaccination including severe allergic reaction, local injection site reaction (swelling, redness, pain), elevated body temperature between 37°C and 38°C or body temperature >38°C, headache, arthralgia, fatigue, or hospitalization within 14 days after vaccination were recorded at all follow-up visits. Biobank samples (serum, plasma) are stored at −80°C at Biobank Graz, located at the Medical University of Graz, for further analysis. Antibody tests were conducted at the D&F Institute of Hygiene, Microbiology and Environmental Medicine at the Medical University of Graz. A CE-marked serological test was used according to the manufacturer's protocols to determine and quantify specific antibodies against SARS-CoV-2. Total immunoglobulin (Ig) was determined using the Roche Elecsys anti-SARS-CoV-2 S electrochemiluminescence immunoassay targeting the RBD of the viral spike protein using a Cobas e 801 analytical unit (Roche Diagnostics GmbH, Mannheim, Germany) with a cut-off for positivity of 0.8 U/mL. According to Roche's protocol[16] no converting factor is needed to calculate binding antibody units per millilitre, which were retrospectively established for harmonization of different assays' results and are traceable to the WHO international standard for anti-SARS-CoV-2 Ig.[17]

In addition, antibody levels, measured 14 to 21 days after the second COVID-19 vaccination in a cohort of 86 healthy people, recruited in a partner study (EudraCT: 2021-001040-10) at the Medical University of Graz, were used for group comparisons.

The study protocol was approved by the ethics committees of the Medical University of Graz (33-366 ex 20/21) and the *Bayerische Landesaerztekammer* (Nr. 21 031) as well as registered at the European Union Drug Regulation Authorities Clinical Trials registry (EudraCT-Number 2021-001459-15). The study was conducted according to the guidelines of Good Clinical Practice and the Declaration of Helsinki. Prior to study inclusion, participants were informed about all study procedures by a physician and provided written informed consent.

2.1 | Statistical analysis

Data were extracted in Microsoft Excel and analysed in Stata version 16 and R studio 1.4.1. Categorical variables were summarized as frequencies and percentages (%). Quantitative variables were summarized as means and standard deviations (±SD). Categorical variables were compared with diabetes groups using chi-squared or Fisher's exact tests, as appropriate. Quantitative variables were compared with diabetes groups using one-way analysis of variance tests. Post-vaccination, side effects were compared between people with type 1 diabetes and type 2 diabetes using chi-squared or Fisher's exacts tests, as appropriate. Anti-SARS-CoV-2 S antibodies were summarized as median with interquartile range (IQR). The median anti-SARS-CoV-2 S antibody levels were compared between diabetes groups and healthy controls using Kruskal-Wallis tests. These group comparisons were adjusted for age and sex using quantile regression and further corrected for multiple comparisons using post-hoc Bonferroni correction, or Wilcoxon's signed-rank test, respectively. The correlation of anti-SARS-CoV-2 S antibodies with quantitative variables was assessed using the Pearson correlation method. The P value of <0.05 was chosen to indicate statistical significance.

3 | RESULTS

We enrolled 161 participants with diabetes between April and June 2021 in the study, of whom 150 were included in the final analysis (Figure S1). Two participants withdrew consent, six participants decided to postpone their vaccination for a longer period after the baseline visit and three participants had positive anti-SARS-CoV-2 S antibodies at baseline without knowingly having had a COVID-19 episode before. (Figure S1). Seventy-five participants had type 1 diabetes (34 of whom were women) with 49 in the HbA1c ≤58 mmol/mol group and 26 in the HbA1c >58 mmol/mol group. In addition, 75 participants had type 2 diabetes (34 of whom were women), with 37 in the HbA1c ≤58 mmol/mol group and 38 in the HbA1c >58 mmol/mol group. Participants with type 2 diabetes were older as compared to those with type 1 diabetes (56.6 ± 9.9 years vs. 41.5 ± 14.5 years; $P < 0.001$) and had a higher prevalence of hypertension, hyperlipidaemia, liver disease and polyneuropathy (all $P < 0.05$). Of all participants, 86% received the BioNTech/Pfizer, 8.7% the Moderna and 5.3% the AstraZeneca vaccine. Vaccine distribution was similar in all four groups of participants with diabetes ($P = 0.542$). The mean time between the baseline visit and the first vaccination was 11 ± 13 days. A full list of the baseline characteristics of all four groups of study participants is provided in Table 1.

3.1 | Healthy control group

For comparison we used a cohort of 86 healthy participants. Of these, 49 (57%) were women and the mean age was 48 ± 11.6 years and the mean body mass index (BMI) 24.2 ± 3.6 kg/m^2. In this group, 96.5% received the Moderna and 3.5% the BioNTech/Pfizer vaccine. All participants in the healthy control group had no detectable anti-SARS-CoV2-S antibodies pre-vaccination and no history of COVID-19.

3.2 | Side effects of vaccination

Three cases of hospitalization occurred after the vaccination. One occurred 24 days after the first vaccination due to peripheral oedema

TABLE 1 Baseline characteristics ($N = 150$)

Variables	All ($N = 150$)	T1DM and HbA1c > 58 mmol/mol ($N = 26$)	T1DM and HbA1c ≤ 58 mmol/mol ($N = 49$)	T2DM and HbA1c > 58 mmol/mol ($N = 38$)	T2DM and HbA1c ≤ 58 mmol/mol ($N = 37$)	*P* value
Age	49.2 ± 14.5	42.7 ± 14.0	40.8 ± 14.8	56.9 ± 9.6	56.3 ± 10.3	<0.001
Sex, *n* (%)						
Female	68 (45.3)	10 (38.5)	24 (49.0)	20 (52.6)	14 (37.8)	0.491
Male	82 (54.7)	16 (61.5)	25 (51.0)	18 (47.4)	23 (62.2)	
BMI, kg/m^2	28.7 (5.6)	27.9 (5.1)	24.6 (3.9)	32.7 (5.3)	30.5 (4.5)	<0.001
Vaccine, *n* (%)						
BioNTech Pfizer	129 (86.0)	24 (92.2)	40 (81.6)	35 (92.1)	30 (81.1)	0.542
Moderna	13 (8.7)	1 (3.9)	5 (10.2)	3 (7.9)	4 (10.8)	
AstraZeneca	8 (5.3)	1 (3.9)	4 (8.2)	0 (0.0)	3 (8.1)	
Duration of diabetes	16.0 ± 12.0	23.6 ± 13.6	18.1 ± 12.9	13.5 ± 9.4	10.6 ± 8.5	<0.001
Diabetes therapy, *n* (%)						
Insulin	104 (69.3)	26 (100.0)	48 (98.0)[a]	21 (55.3)	9 (24.3)	<0.001
Metformin	56 (37.3)	-	-	28 (73.7)	28 (75.7)	0.843
DPP-4 inhibitors	19 (12.7)	-	-	12 (31.6)	7 (18.9)	0.208
SGLT2 inhibitors	27 (18.0)	-	-	16 (42.1)	11 (29.7)	0.264
GLP-1RAs	15 (10.0)	-	-	8 (21.1)	7 (18.9)	0.817
Comorbidity						
Hypertension, *n* (%)	66 (44.0)	7 (26.9)	9 (18.4)	25 (65.8)	25 (67.6)	<0.001
Coronary heart disease, *n* (%)	14 (9.3)	1 (3.9)	2 (4.1)	6 (15.8)	5 (13.5)	0.160
Myocardial infarction, *n* (%)	7 (4.7)	0 (0.0)	1 (2.0)	4 (10.5)	2 (5.4)	0.209
Stroke, *n* (%)	5 (3.3)	1 (3.9)	0 (0.0)	3 (7.9)	1 (2.7)	0.169
Heart failure, *n* (%)	4 (2.7)	0 (0.0)	1 (2.0)	2 (5.3)	1 (2.7)	0.830
PAD, *n* (%)	10 (6.7)	0 (0.0)	2 (4.1)	4 (10.5)	4 (11.1)	0.232
PTCA/CABG, *n* (%)	7 (4.7)	0 (0.0)	1 (2.0)	3 (7.9)	3 (8.1)	0.327
CVD, *n* (%)	15 (10.0)	1 (3.9)	2 (4.1)	6 (15.8)	6 (16.2)	0.111
Hyperlipidaemia, *n* (%)	70 (46.7)	11 (42.3)	12 (24.5)	25 (65.8)	22 (59.5)	<0.001
Liver disease, *n* (%)	23 (15.3)	1 (3.9)	1 (2.0)	9 (23.7)	12 (32.4)	<0.001
History of cancer, *n* (%)	8 (5.3)	2 (7.7)	0 (0.0)	2 (5.3)	4 (10.8)	0.083
Microvascular complications						
Retinopathy, *n* (%)	21 (14.0)	7 (26.9)	6 (12.2)	5 (13.2)	3 (8.1)	0.215
Polyneuropathy, *n* (%)	29 (19.3)	3 (11.5)	3 (6.1)	15 (39.5)	8 (21.6)	0.001
Laboratory values						
HbA1c, mmol/mol	56.7 ± 12.5	67.9 ± 9.8	49.3 ± 6.7	67.8 ± 9.3	47.5 ± 6.9	<0.001
eGFR, mL/min/1.73m^2	92.5 ± 20.9	96.3 ± 26.5	101.5 ± 17.5	80.6 ± 18.9	89.6 ± 15.9	<0.001
HDL cholesterol, mmol/L	1.51 ± 0.50	1.63 ± 0.57	1.79 ± 0.44	1.22 ± 0.34	1.37 ± 0.47	<0.001
LDL cholesterol, mmol/L	2.36 ± 0.88	2.46 ± 0.84	2.43 ± 0.75	2.2 ± 0.95	2.38 ± 1.02	0.633
Triglycerides, mmol/L	1.32 ± 1.14	1.13 ± 0.53	2.01 ± 1.88	0.89 ± 0.35	1.3 ± 0.74	0.121

Note: Qualitative variables are presented as frequencies and percentages (%). Quantitative variables are presented as means and standard deviations (±SD). Chi-squared or Fischer's exact tests were applied to compare qualitative variables with diabetes groups. One-way analysis of variance tests were applied to compare quantitative variables with diabetes groups.

Abbreviations: CVD, cardiovascular disease; DPP-4, dipeptidyl peptidase-4; GLP-1RA, glucagon-like peptide-1 receptor agonist; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAD, peripheral artery disease; PTCA, percutaneous transluminal coronary angiography, CABG, coronary artery bypass graft; SGLT2, sodium glucose cotransporter-2; T1DM, type 1 diabetes; T2DM, type 2 diabetes.

[a]One participant was recently diagnosed with type 1 diabetes and off insulin at the time of enrolment (honeymoon period).

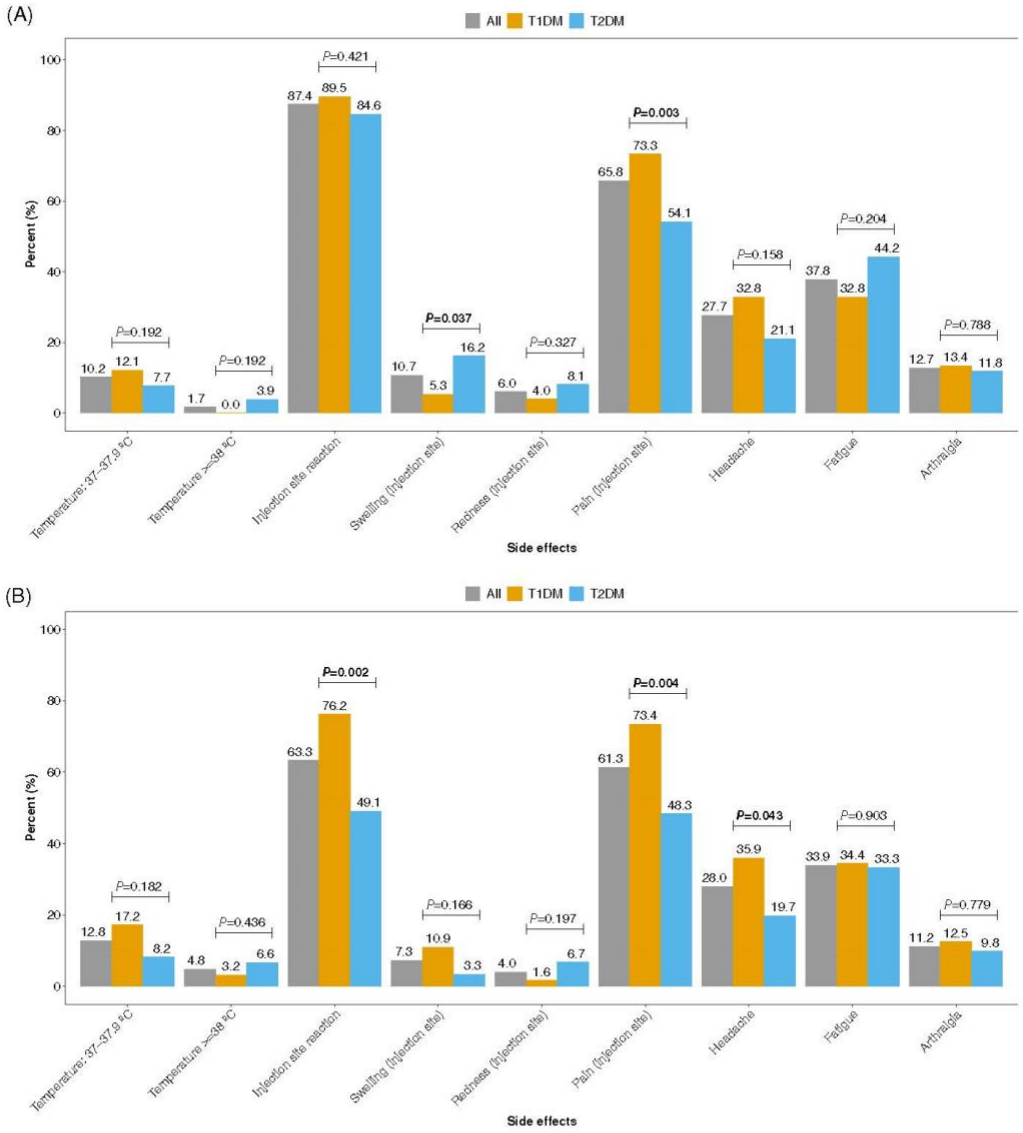

FIGURE 1 Side effects after vaccination, overall and by type of diabetes: A, after vaccination 1 and B, after vaccination 2. T1DM, type 1 diabetes; T2DM, type 2 diabetes

and chronic heart failure; chronic heart failure was a preexisting condition in this patient. The second hospitalization took place 12 days after the second vaccination due to atrioventricular block grade 3 with subsequent pacemaker implantation, and the third hospitalization occurred due to a miscarriage after 10 weeks of pregnancy. Conception was estimated at 2 weeks after the first vaccination. No cases of severe allergic reactions were recorded throughout the study. The most common side effects were injection site reactions, occurring in 87.4% of all participants after the first and 63.3% after the second dose, with a significantly lower rate in people with type 2 diabetes at the latter vaccination. Fever was rarely reported in any of the groups (for a detailed overview see Figure 1).

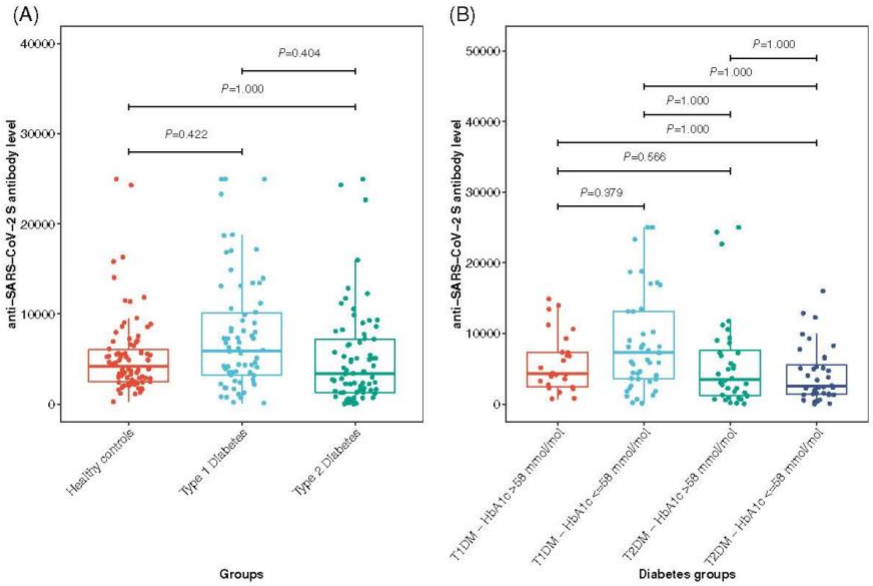

FIGURE 2 A, Comparison of anti-SARS-CoV-2-S antibodies between participants with diabetes and healthy controls after the second vaccination. B, Comparison of anti-SARS-CoV-2-S antibodies in people with type 1 (T1DM) and type 2 diabetes (T2DM) and a glycated haemoglobin (HbA1c) level of ≤58 mmol/mol or >58 mmol/mol, respectively. *P* values are adjusted for age and sex using quantile regression and for multiple comparison using Bonferroni correction

3.3 | Antibody response

At 7 to 14 days after the first vaccination, 52.7% of the participants with type 1 diabetes and 48.0% of those with type 2 diabetes had anti-SARS-CoV2-S RBD antibodies above the detection limit of 0.8, with low median levels of 1.1 (IQR 8.1) and 0.3 (IQR 2.4), respectively. When we analysed the antibody data measured after the second vaccination, we first pooled the two groups of participants with type 1 diabetes and the two groups of participants with type 2 diabetes (HbA1c ≤58 mmol/mol and >58 mmol/mol), respectively, and compared these against the healthy controls. In the unadjusted analyses we observed the highest antibody levels after second vaccination in people with type 1 diabetes ($P = 0.022$ vs. healthy controls and $P = 0.013$ vs. people with type 2 diabetes). However, these significant differences were no longer present after adjustment for age, sex and correction for multiple comparisons (Figure 2A).

In addition, we investigated the impact of type of diabetes and glycaemic control on antibody response after COVID-19 vaccination. In the group comparison corrected for multiple comparisons only, people with type 1 diabetes and an HbA1c ≤58 mmol/mol had no statistically significant different antibody levels as compared to people with type 1 diabetes and an HbA1c >58 mmol/mol ($P = 0.249$). In comparison to participants with type 2 diabetes, those with type 1 diabetes and an HbA1c ≤58 mmol/mol had significantly higher antibody levels ($P = 0.034$ for type 2 diabetes and HbA1c ≤58 mmol/mol and $P = 0.003$ for type 2 diabetes and HbA1c >58 mmol/mol, respectively). After adjusting for age and sex and correcting for multiple comparisons, a significant difference between the groups was no longer observed (Figure 2B). The results did not change after also adjusting for BMI and estimated glomerular filtration rate (eGFR; no significant difference between the glycaemic groups) (Figure 3).

We also performed a sensitivity analysis by using an HbA1c of 53 mmol/mol (7.0%) as the cut-off for the glycaemic groups. In the age- and sex-adjusted quantile regression analysis for the entire diabetes group (type 1 and type 2 diabetes together) the HbA1c category was not a predictor of anti-SARS-CoV2-S antibody levels ($P = 0.535$). Likewise, when comparing the antibody levels of the newly formed four groups (type 1 diabetes with an HbA1c <53 mmol/mol and type 1 diabetes with an HbA1c ≥53 mmol/mol and type 2 diabetes with an HbA1c <53 mmol/mol and type 2 diabetes with an HbA1c ≥53 mmol/mol), no significant difference between the groups was observed when adjusted for age and sex (all $P > 0.1$). In people with type 2 diabetes, insulin treatment was not associated with the antibody response observed in adjusted quantile regression analysis ($P = 0.165$).

3.4 | Clinical characteristics and antibody response

We pooled all participants with diabetes to perform correlation analyses, in which age was moderately to strongly correlated with anti-SARS-CoV-2 S RBD antibody levels ($r = -0.45$, $P < 0.001$), an association that was more pronounced in participants with type 1 diabetes (−0.53,

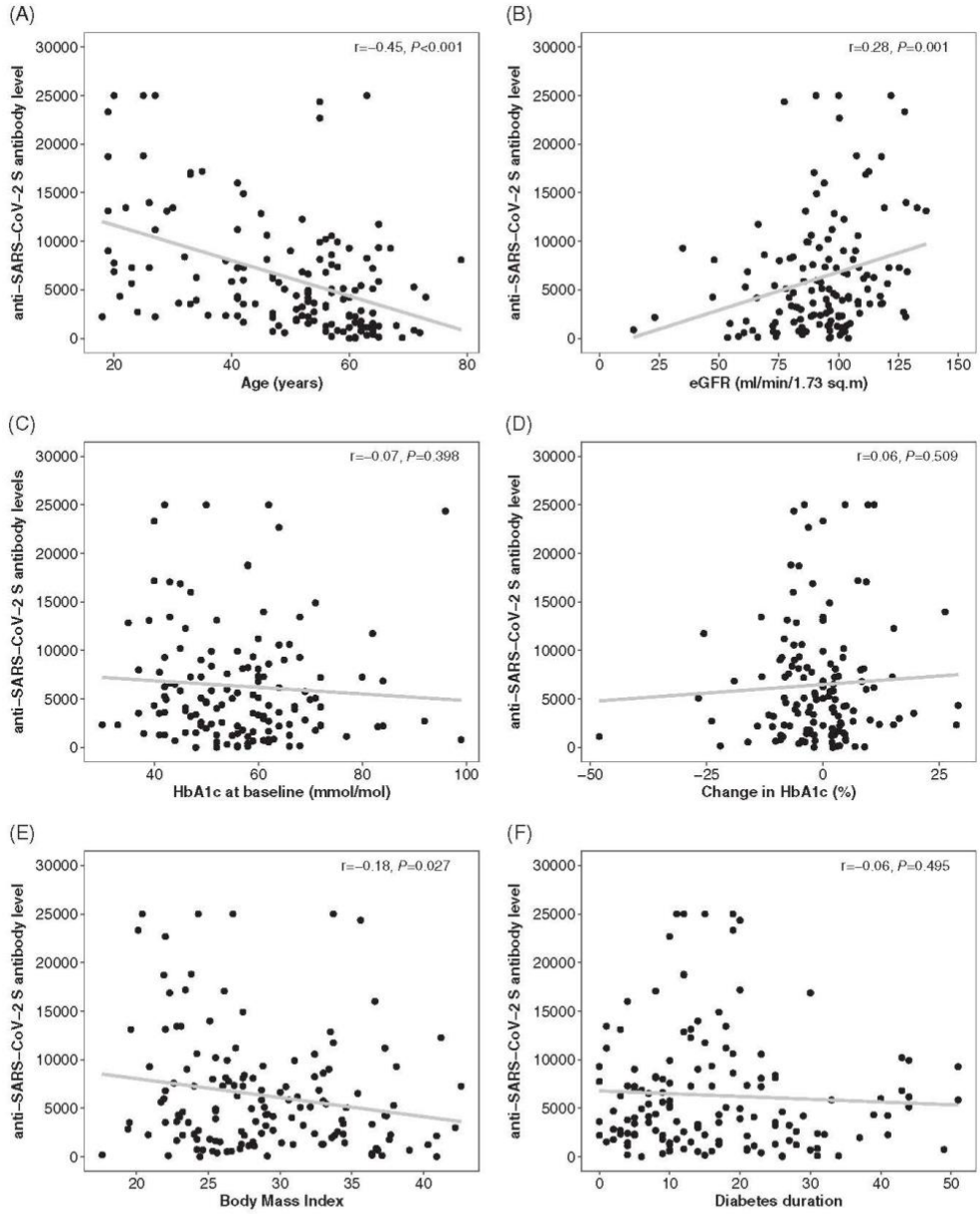

FIGURE 3 Correlation plots for selected clinical characteristics. eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; *r*, Pearson's correlation coefficient. *P* values are for Pearson's correlation

$P < 0.001$) than in those with type 2 diabetes ($r = -0.20$, $P = .087$). eGFR was also directly associated with levels of anti-SARS-CoV-2 S RBD antibodies ($r = 0.28$, $P = 0.001$), while no correlation was observed with either HbA1c levels at baseline ($r = -0.07$, $P = 0.398$) or with changes of HbA1c levels between baseline and the follow-up visit after the second vaccination ($r = 0.06$, $P = 0.509$) as a measure of change in glycaemic

control between the vaccinations. BMI was weakly and inversely correlated with humoral immune response ($r = -0.18$, $P = 0.027$). Gender and diabetes duration had no impact on the antibody response.

If participants developed an elevated body temperature (>37.0°C) after the second vaccination, the antibody response appeared to be more pronounced ($P = 0.036$) as compared to in those without such an increase in body temperature (Figure S3).

4 | DISCUSSION

This study demonstrated that people with type 1 and type 2 diabetes display a humoral immune response to COVID-19 vaccination, measured according to SARS-CoV-2 S RBD antibodies, that is comparable to that of healthy controls. While unadjusted analyses suggested higher antibody levels in people with type 1 diabetes and an HbA1c ≤58 mmol/mol, no significant difference persists after adjustment for age, sex and correction for multiple comparisons. Our correlation analyses also suggest that age and eGFR are predictors for antibody levels after COVID-19 vaccination, while HbA1c levels are not.

These study results are in contrast to a recently published observational study (CAVEAT study) that demonstrated a lower antibody response to COVID-19 vaccination in people with type 2 diabetes having an HbA1c above 53 mmol/mol (7.0%).[18] Although we predefined a cut-off of 58 mmol/mol (7.5%) for this analysis, the mean HbA1c levels observed in our cohort in the two groups are comparable to those in the CAVEAT study. When we analysed our data using an HbA1c cut-off of 53 mmol/mol (7.0%) the results remained unchanged.

One explanation for the divergent results might be that the CAVEAT study used the GenScript SARS-CoV-2 surrogate virus neutralization test while in this study we used the Roche Elecsys anti-SARS CoV-2 S assay targeting the RBD. However, in direct comparison studies, both assays have demonstrated good correlation with each other, with an agreement rate of approximately 90%.[19]

Our study suggests that age is a major determinant of humoral immune response to a COVID-19 vaccination. This is in agreement with previous studies showing that elderly people not only exhibit a lower antibody response to different types of vaccines such as diphtheria, hepatitis A, hepatitis B, pneumococcal polysaccharide vaccine, tick-borne encephalitis, tetanus or trivalent influenza vaccine, they also display a more rapid waning of antibodies.[20]

In addition, the eGFR in our study was directly associated with the level of anti-SARS-CoV-2 S antibodies. This is in line with previous data demonstrating that seroconversion rates after HBV vaccination decreases with lower kidney function from 95% in healthy subjects to 40% to 50% in people with CKD stages 3 to 4.[21]

As in studies with hepatitis vaccines,[22] we were also able to show a significant inverse association of BMI with anti-SARS-CoV-2 S RBD antibodies. However, in contrast to the hepatitis vaccination study, the correlation in our dataset was rather weak ($r = -0.19$, $P = 0.027$). Also, no correlation was found with diabetes duration in our study ($r = -0.06$, $P = 0.495$).

This study has some limitations. We aimed to recruit 40 participants into each subgroup of people with diabetes, a number which, despite large efforts, was not reached for those with type 1 diabetes and an HbA1c >58 mmol/mol. In addition, in this study we focused on the humoral immune response against the RBD of the spike protein only and did not further investigate the cellular immune response after the vaccination. However, previous studies have shown that neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infections.[23] Although a number of studies have demonstrated that the widely used Roche Elecsys anti-SARS-CoV-2 S immunoassay correlates with neutralizing anti-SARS-CoV-2 antibody assays,[24-27] we cannot completely exclude the possibility that results with other assays measuring directly neutralizing activity would differ in our cohorts.

As our study is still ongoing, with follow-up visits planned before and after a potential 3rd vaccination and/or 12 months after the baseline visit, data on cellular immune response will be available at future visits.

Another limitation of our study is that 142 participants (94.6%) received an mRNA-based vaccine. However, since we performed a real-world cohort study within the setting of the national vaccination strategy of Austria and Germany, the observed distribution of vaccines represents the actual distribution in these countries in people with diabetes. Moving forward, in Austria only the mRNA-based vaccine is available for the third vaccination. We would like to note the further limitation, that in the healthy control group, 96.5% received the Moderna vaccine, while 86% of the participants with diabetes received the BioNtech-Pfizer and only 8.6% the Moderna vaccine. Previous studies in other cohorts showed that there appears to be a difference in the immune response between the two mRNA vaccines, with Moderna leading to higher antibody levels.[28,29] However, people in the healthy control group were not found to have higher anti-SARS-CoV2-S antibody levels in our study, despite this potential bias.

The COVAC-DM study demonstrated similar humoral immune response to COVID-19 vaccination in people with type 1 and type 2 diabetes and healthy controls when results were adjusted for age, which, together with renal function, had a significant impact on antibody response in our study cohort. Additional information on cellular immune response and further follow-up of participants in our and other clinical trials will help to clarify the full picture regarding vaccination effects and trajectories of antibody levels after COVID-19 vaccination in people with diabetes and to provide more specific definitions of re-vaccination intervals depending on patient characteristics.

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CONFLICT OF INTEREST

None of the authors have conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Harald Sourij, Norbert J. Tripolt, Faisal Aziz, Ivo Steinmetz and Caren Sourij designed the study. Caren Sourij and Harald Sourij drafted the first version of the manuscript. Harald Kojzar, Peter N. Pferschy, Nadine Wachsmuth and Norbert J. Tripolt performed the data preparation. Faisal Aziz performed statistics and created the figures. Caren Sourij, Felix Aberer, Anna M. Obermayer, Harald Kojzar, Peter N. Pferschy, Farah Abbas, Julia K. Mader, Max L. Eckstein, Alexander Müller, Jacqueline Lenz, Gerhard Cvirn, Nandu Goswami, Michaela Steinberger, Lisa Knoll, Nazanin Sareban, performed the subject recruitment and were in charge of the conduction of study visits. Barbara Kleinhappl and Patrick Forstner performed antibody measurements. Ivo Steinmetz supervised antibody measurements. Barbara Prietl performed lab measurements. Robert Krause, Martin Stradner, Peter Schlenke supervised and designed the healthy control study. Data on healthy subjects was provided by Martin Stradner. Susanne Kaser acted as principal investigator of the participating study centre in Innsbruck. Othmar Moser was responsible for the performance of the study at the centre in Bayreuth. All authors have carefully revised the manuscript, agreed to the submission of the latest version and sufficiently contributed to this work. The samples/data used for this project have been provided by Biobank Graz of the Medical University of Graz, Austria.

All authors critically revised the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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7.6. Appendix 6

Impact of COVID-19 Vaccination on Glycemia in Individuals With Type 1 and Type 2 Diabetes: Substudy of the COVAC-DM Study

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As people with diabetes are considered a high-risk population in the coronavirus disease 2019 (COVID-19) pandemic scenario, they are globally prioritized in COVID-19 vaccine policy. To date, in most industrialized countries, people with diabetes have already been offered the opportunity to receive a vaccine. Nonetheless, concerns exist about vaccine-induced dysglycemia among people living with diabetes, potentially holding them back from getting vaccinated. Immune responses can trigger alterations in insulin sensitivity, potentially increasing insulin requirements due to inflammation, humoral, and cellular immune responses (1). Assuming that natural immunization (infection) triggers (patho)physiological patterns similar to those of pharmacological immunization (vaccination), one can speculate that diabetes management also deteriorates after the vaccination (2), as proinflammatory cytokines increase insulin resistance (3).

Considering this, we aimed to investigate the short-term effects of COVID-19 vaccination on the time spent in different glycemic ranges, assessed by continuous glucose monitoring (CGM) in people with type 1 and type 2 diabetes.

From April to June 2021, a total of 161 individuals were enrolled in the multicenter prospective Immune Response to COVID-19 Vaccination in People with Diabetes Mellitus—COVAC-DM Study (EudraCT2021-001459-15), of whom 74 participants had sufficient CGM data (at least 90%) available around their first COVID-19 vaccination to be included in the present substudy. Fifty-eight individuals had type 1 diabetes (mean age 39.5 ± 14.1 years; mean HbA_{1c} 57 ± 12 mol/mol), and 16 had type 2 diabetes (mean age 60.6 ± 6.2 years; mean HbA_{1c} 63 ± 11 mmol/mol). Of those with type 1 diabetes, 22 (37.9%) were on continuous subcutaneous insulin infusion (total daily bolus insulin, as median [interquartile range], 17 [11]; basal insulin 23 [15] IU) and 36 (62.1%) were on multiple daily insulin injections (MDI) (total daily bolus insulin 19 [12]; basal insulin 19 [12] IU). Fifteen people with type 2 diabetes were on MDI (total daily bolus insulin 18 [30]; basal insulin 28 [20] IU), and one participant was on oral glucose-lowering drugs. The majority of participants received an mRNA-based vaccine (87% BioNTech Pfizer and 6% Moderna vs. 7% AstraZeneca). Data from the CGM were analyzed from 2 days prior until 3 days after the first dose of vaccination for time spent in different glycemic ranges (4).

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F.Abe., O.M., and F.Az. contributed equally to this work.

*A complete list of the COVAC-DM Study Group members can be found in the supplementary material online.

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Furthermore, we investigated prospectively whether the presence and severity of self-reported typical side effects and elevated body temperature following the first COVID-19 vaccination alter glycemia and whether bolus insulin dosing behavior and carbohydrate intake were influenced in response to the vaccination.

To assess time spent in glycemic ranges in relation to specific side effects, we created a simple score: the presence of any side effect, including headache, body ache, fatigue, and any injection site reaction after vaccination added one point, as did the presence of elevated body temperature of >37°C following the vaccine. If two of the criteria (at least one side effect and elevated body temperature) were met, participants received two points; if none of the criteria were met, participants received zero points. We allocated each day of each participant with available CGM data a side effect score according to the definition described above. Analyses were defined in a statistical analysis plan ahead of study completion.

In total, 49,200 CGM data points were available. For both people with type 1 diabetes (P = 0.962) and type 2 diabetes (P = 0.704), no significant differences were found for the time in range (TIR) (70 – 180 mg/dL) over the course of the vaccination from 2 days prior to receiving the vaccination until 3 days afterward (Fig. 1*A* and *B*). Likewise, the time below range (TBR) (<70 mg/dL; type 1 diabetes, P = 0.952; type 2 diabetes, P = 0.704) and the time above range (TAR) (>180 mg/dL; type 1 diabetes, P = 0.941; type 2 diabetes, P = 0.715) did not change around the COVID-19 vaccination.

The bolus insulin dose was not adjusted around the vaccination in people with either type 1 (P = 0.578) or type 2 (P = 0.346) diabetes. Similar results were seen when assessing carbohydrate intake, detailing no significant difference in carbohydrate intake in people with type 1 (P = 0.092) and type 2 (P = 0.958) diabetes around the COVID-19 vaccination.

Thirty-five percent of the available CGM days post-vaccination with complete side effect information were spent with side effect score 0, 58% with side effect score 1, and 7% with side effect score 2. When separating days of people with type 1 and type 2 diabetes based on the side effect score, those with type 1 diabetes spent significantly less TIR on days with an increased side effect score (1 or 2) than on days with a side effect score of 0 (P = 0.033 using Wilcoxon rank sum test). This finding was confirmed, as the TAR was significantly higher on days with a side effect score of >0 (P = 0.043 using Wilcoxon rank sum test). The side effects had no significant impact on the TBR in people with type 1 diabetes (P = 0.925). In people with type 2 diabetes, the side effect score had no influence on the TIR (P = 0.865), TAR (P = 0.856), and TBR (P = 0.081) (Fig. 1*C* and *D*).

Glycemic variability, measured as coefficient of variation, was not significantly different with respect to side effect score for either type 1 (P = 0.206) or type 2 (P = 0.501) diabetes (data not shown).

Our study is not without limitations. In particular, our sample size with CGM data in people with type 2 diabetes is rather small; hence, our results cannot be transferred to the entire population of people with diabetes. Moreover, all but one participant of the substudy were on continuous subcutaneous insulin infusion or MDI; therefore, we

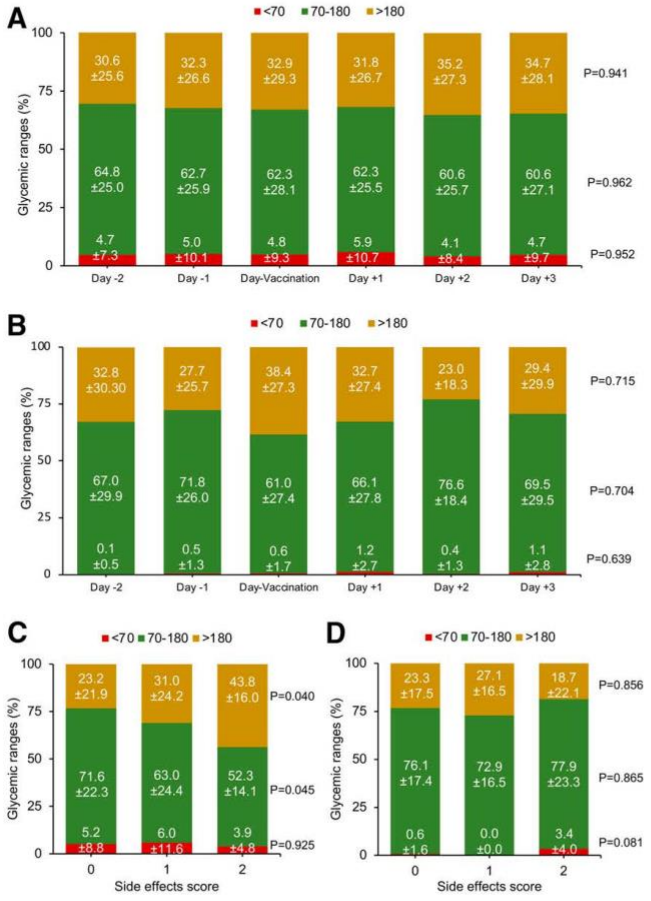

Figure 1—TIR in response to the COVID-19 vaccination in people with type 1 diabetes (*A*) and type 2 diabetes (*B*). Glycemic ranges were based on the side effect score for people with type 1 diabetes (*C*) and type 2 diabetes (*D*). Data were analyzed according to "score-days." Glycemic ranges are given in mg/dL.

cannot comment on the impact of glycemic excursions in people with type 2 diabetes treated with oral glucose-lowering agents or diet only.

Our data revealed that COVID-19 vaccination per se did not change glycemic control in people with diabetes. Of note, on days on which side effects were present, a deterioration of glycemia was observed in people with type 1 diabetes. While this observation should be further investigated in larger studies, it can be considered when health care professionals inform their patients about potential glycemic aberrations in response to COVID-19 immunization.

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