Biomarkers for Diabetes Mellitus in advanced Peripheral Arterial Disease

Diagnostic performance and outcome prediction of HbA1c, fasting plasma glucose and the oral glucose tolerance test

Iren Drange Hjellestad

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Scientific Environment

This PhD project is an interdisciplinary cooperation between the Department of Vascular Surgery at Haukeland University Hospital, the Department of Medicine at Haukeland University Hospital, the Department of Clinical Sciences at the University of Bergen, and the Hormone Laboratory, Department of Laboratory Medicine and Pathology, Haukeland University Hospital, Bergen, Norway. The Western Norway Health-Authority funded the research project. The PhD work was funded by a 12 month 50% temporary research position at the Department of Medicine at Haukeland University Hospital, Bergen, Norway, in 2012 and a 20% research position at the Hormone Laboratory at Haukeland University Hospital, Bergen, Norway, from June 2016 until March 2018 and from august 2018 until present. In 2017, the PhD candidate received a grant from the Norwegian Diabetes Association.

Main supervisor for the PhD project was Torbjörn Jonung, MD, Dr. Med, Professor of Vascular Surgery at the Department of Clinical Sciences at the University of Bergen, Norway, and at the Department of Vascular Surgery at Haukeland University Hospital, Bergen, Norway.

Co-supervisors were Eirik Søfteland, MD, PhD of Endocrinology at the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway, and Eystein Husebye, MD, Dr. Med, Professor of Endocrinology at the Department of Clinical Sciences at University of Bergen, Norway, and at the Department of Medicine at Haukeland University Hospital, Bergen, Norway.

Collaborators: Roy Miodini Nilsen, PhD, statistician at Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway, and Karl Ove Hufthammer, PhD, statistician at Centre for Clinical Research, Haukeland University Hospital Bergen, Norway.

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Contents

Abbreviations

AAA	abdominal aortic aneurysm
ABI	ankle-brachial index
ADA	American Diabetes Association
ADAG	A1C-derived Average Glucose
AGE	advanced glycation end products
AUC	area under the curve
BMI	body mass index
CAD	coronary artery disease
CI	confidence interval
CTA	computed tomographic angiography
CV	coefficient of variation
CVD	cerebrovascular disease
DCCT	Diabetes Control and Complications Trial
DECODE	Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe
DM	diabetes mellitus
ESC	European Society of Cardiology
FFA	free fatty acid
Fin-D2D	National type 2 diabetes prevention programme in Finland
FPG	fasting plasma glucose
GLP-1	glucagon-like peptide 1
GP	General Practitioner
Hb	Haemoglobin
HbA_{1c}	Haemoglobin A_{1c}/ glycosylated haemoglobin
HR	hazard ratio

IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IOD	iliac occlusive disease
LADA	latent autoimmune diabetes of adult
LDL	low-density lipoprotein
MODY	Maturity onset diabetes in the young
NGSP	National Glycohaemoglobin Standardization Program
NOKLUS	Norwegian Quality Improvement of Laboratory Examinations
NORKAR	Norwegian Cardiovascular Disease Registry
OGTT	oral glucose tolerance test
PAD	peripheral arterial disease
ROC	receiver operating characteristic
ROS	reactive oxygen species
SGLT-2	sodium-glucose cotransporter 2
TASC II	The Trans-Atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease
WHO	World Health Organization

Abstract

Background

At present, the diagnosis of diabetes mellitus (DM) is based on the result from either of three tests; HbA_{1c}, fasting plasma glucose (FPG) or 2-hour post glucose-load value following an oral glucose tolerance test (OGTT). Although DM is one of the major risk factors for peripheral arterial disease (PAD), undiagnosed DM is common in PAD patients. Also, epidemiological studies have shown that FPG, the OGTT and HbA_{1c} largely classify different patients as having DM and pre-DM.

Studies have reported a negative association of abdominal aortic aneurysms (AAA) with DM. However, inconsistent results regarding long-term survival in both AAA patient of normal and abnormal glucose status are reported.

Aims and Methods

This was a prospective cohort study of patients with advanced vascular disease. The vascular pathologies were carotid stenosis, iliac occlusive disease (IOD), infra-inguinal occlusive disease and AAA.

In patients of unknown glycaemic status, the aim was to evaluate the performance of HbA_{1c} as a diagnostic test for DM when using the OGTT as the gold standard (Paper I) and to explore the association of HbA_{1c} levels, FPG values and OGTT results with long-term survival (Paper III).

In Paper II, data from 66 AAA patients was used to investigate the prevalence of DM and pre-DM. Further, to evaluate the association of glycaemic status assessed by $HbA_{1c,}$ FPG and OGTT measurements with long-term survival in these patients.

Results

Paper I

The prevalence of newly diagnosed DM was 12% and that of pre-DM was 33% based on OGTT results. An HbA_{1c} value of ≥ 48mmol/mol (6.5%) detected DM with 45.5% sensitivity and 90% specificity. The total prevalence of DM and pre-DM was

higher based on HbA_{1c} values than based on the OGTTs. AUC for the association of HbA_{1c} with the OGTT and FPG as diagnostic parameters for DM was 0.73 (95% CI 0.63-0.84).

Paper II

The total prevalence of known and newly diagnosed DM in 66 AAA patients were 23% based on OGTT results. The prevalence of newly diagnosed DM in 58 AAA patients of unknown glycaemic status at baseline was 12% by OGTT results. HbA_{1c} values classified DM patients according to OGTT results with 43% sensitivity and 90% specificity. The prevalence of pre-DM was 43% based on OGTT results and 72% based on HbA_{1c} values.

In 5.9 years follow-up, all-cause mortality in AAA patients of unknown glycaemic status was 43%. HbA_{1c} was a significant independent predictor for mortality in the DM category, adjusted Hazard Ratio (HR) 6.35, (95% CI 1.49-27.1); p=0.01.

Paper III

Nine-years all-cause mortality in vascular surgery patients of unknown glycaemic status was 40%. HbA_{1c} was an independent predictor for mortality, adjusted HR 1.54, (95% CI 1.03 – 2.32); p=0.04. The OGTT was not a predictor of long-term mortality.

Conclusion

Vascular surgery patients have a high prevalence of undiagnosed DM and pre-DM.

The OGTT and HbA_{1c} largely identify different patient groups as having DM and pre-DM. The number of vascular surgery patients having DM and pre-DM is higher based on HbA_{1c} values than based on OGTT measurements.

In patients with advanced vascular disease and of unknown glycaemic status, HbA_{1c} may be a useful marker to identify patients at high risk of a reduced survival rate following surgical treatment for PAD.

List of Publications

Paper I **Hjellestad ID**, Astor MC, Nilsen RM, Softeland E, Jonung T. HbA1c versus oral glucose tolerance test as a method to diagnose diabetes mellitus in vascular surgery patients. Cardiovasc Diabetol 2013 May 25;12(1):79.

Paper II **Hjellestad ID**, Søfteland E, Nilsen RM, Husebye, E, Jonung T. Abdominal aortic aneurysms – glycaemic status and mortality. Journal of Diabetes and Its Complications 30 (2016) 438–443.

Paper III **Hjellestad ID**, Søfteland E, Husebye ES, Jonung T. HbA1c predicts long-term postoperative mortality in patients with unknown glycemic status at admission for vascular surgery: An exploratory study. Journal of Diabetes. 2018;1–11.

1. Introduction

1.1 A Historical Perspective

Atherosclerosis is a fundamental process of vascular ageing in humans[1]. Palaeontological studies have identified atherosclerosis in ancient mummies[2, 3]. In addition, paleo-genetic studies of Ötzi, the Tyrolean glacier-mummy (3300 BC), identified a genetic predisposition for cardiovascular diseases (CVD)[3]. Ageing and genetic predisposition are strong non-modifiable risk factors for atherosclerosis[4, 5]. Although atherosclerosis is part of the normal ageing in humans, some people develop symptomatic atherosclerotic disease whilst others do not. This may largely be explained by modifiable risk factors for atherosclerosis; diabetes mellitus (DM), hypertension, dyslipidemia, obesity and smoking[6-9].

The earliest reference to DM, reported as polyuria, was included in The Eber's Papyrus dated 1550 BC[10]. Despite the ancient recognition of a polyuria-related disease, the first known description and attribution of DM was recorded in years 0-100 AD[10]. At that time, DM was thought to be a disease of the kidneys. It was not until the 19th century that DM was defined as a pancreatic disease following the observation that removal of pancreas in dogs caused DM[11]. Thus, for millennia, DM persisted to be a rapidly evolving disease with a fatal outcome[10, 11].

The first milestone in the treatment of DM was the discovery of insulin in the 1920's[12]. After the implementation of insulin followed by Sulfonylurea and Metformin in the 1950's and -60's and thereafter the exponentially increasing treatment possibilities for DM[13], DM patients have reached almost the same life expectancy as non-diabetic individuals. Hence, DM related micro- and macro-vascular complications were introduced to clinicians as a new problem in patients with DM following improved treatment possibilities.

Throughout the following decades, numerous studies that aimed to investigate and describe the increased risk of vascular complications in diabetes mellitus reported an increased risk for both micro- and macrovascular complications[14-16].

Following studies showing that hyperglycaemia was associated with vascular complications, several intervention-studies aimed to evaluate if glucose lowering treatment aiming towards normalization of glucose levels would be protective of micro- and macrovascular complications[17-23].

In parallel with clinical studies on DM treatment, increasing evidence for prolonged anti-platelet therapy in vascular diseases emerged through the 1980's and 1990's and became a cornerstone in medical treatment of vascular disease[24]. Further, the link between atherosclerosis and dyslipidemia was established through intensive research during the 20th century followed by numerous intervention trials showing cholesterol lowering effect and prevention of vascular events and cardiovascular death[25-27].

The impact of multiple risk factors on cardiovascular related death in DM patients was reported in the early 1990's[28]. The Diabetes Mellitus Insulin-glucose Infusion in Acute Myocardial Infarction (DIGAMI 1) study was performed in 1990-1993 in the early era of preventive medical treatment for vascular disease and reported a reduction in mortality for all treatment groups as well as markedly improved survival in the insulin infusion group[29]. The reduced mortality-rate following myocardial infarction in that study may be explained by the increased use of platelet inhibitors and antihypertensive medication.

Recent intervention studies on the effect of Glucagon-like Peptide 1 (GLP-1) analogues and Sodium-Glucose Cotransporter 2 (SGLT-2) inhibitors have brought new advances and hopes in DM treatment by reducing cardiovascular events and cardiovascular related death in DM patients[30-33]. Anti-atherogenic effect due to reduced vascular inflammation is proposed as one possible mechanism for the benefit on cardiovascular outcome. However, caution should be made for the reported adverse effects, in particular the increased risk of ketoacidosis and amputations, related to treatment with sodium-glucose-transporter (SGLT-2) inhibitors[30, 31]. This emphasizes the need for personalized treatment in patients with DM and peripheral arterial disease (PAD).

1.2 Epidemiology

Diabetes mellitus prevalence is increasing both nationally and globally causing an increased disease burden worldwide[34]. The World Health Organization (WHO) estimated the global overall prevalence of DM to be approximately 8.5% in 2014[35], which represents about a four-fold increase in DM prevalence over the past three decades. In a recent update, the Norwegian health authorities estimated that approximately 4.7% of the Norwegian population have DM, of which about 1/10 have DM Type 1 and remaining DM Type 2 or other forms[36].

The prevalence of peripheral occlusive disease is reported to be 15-30%, increasing with advancing age and higher in at risk populations compared to in general populations[37-39]. Screening-studies have reported a prevalence of abdominal aortic aneurysms (AAA) of 4-9% in men and 0.5-2% in women[40,41] whereas the prevalence of carotid stenosis is approximately 4%[42]. One third to half of PAD patients are asymptomatic and therefore untreated[38, 43-45]. As a result, occlusive disease is frequently present before symptoms occur. Also, asymptomatic PAD patients carry a high risk for mortality and vascular events[46].

In contrast to the increase in DM prevalence, a decline in time trends for the prevalence of cardiovascular diseases and cardiovascular-related deaths is reported[37, 47-49]. Reduction in smoking rates, improved surgical treatment, and increase in the rates of risk factor treatment are suggested explanatory factors.

Also, a decline in AAA related mortality is seen in countries with falling smoking rates. Screening-programs, increased rates of elective AAA repair and improved outcome following AAA repair are suggested to be explanatory factors[50].

Life expectancy in Norway has increased with 22 months over the past decade. A high DM prevalence accompanied by an increase in life expectancy has resulted in a 14.5% increase in disease burden related to DM[36]. In addition, DM and vascular diseases largely affect persons of working age, which may have implications on health services and health economy[51].

1.3 DIABETES – Pathophysiology, Definition and Classification

1.3.1 Pathophysiology – A Brief Overview

DM occurs due to an imbalance in glucose homeostasis following insufficient insulin secretion and/or action[52]. It is characterized by hyperglycaemia and disturbances in protein-, lipid- and carbohydrate metabolism[53]. The understanding of DM pathophysiology has changed markedly since Sir Banting's discovery of insulin and the then conception of an insulin-deficient state as the single pathology in DM. Research has contributed to an extended understanding of DM as a set of metabolic disorders with various genetic and environmental causes[54]. At present, eleven mediating pathways of hyperglycaemia are known, including the influence of numerous hormones and their interactions with target tissues (Fig. 1)[54].

Reduced beta cell mass and function leading to defective insulin secretion is the central element in DM pathophysiology (Fig. 1, Step 1). Insulin resistance in muscle, liver and adipose tissue is a main contributor to beta-cell dysfunction (Fig. 1, step 4-6). Additional contributors are altered gut microbiota and low-grade systemic inflammation (Fig. 1, Steps 8 and 9). Also, through impaired appetite regulation, cerebral insulin resistance and inflammation-induced neuronal damage has been suggested as pathophysiological mechanisms for hyperglycaemia[55, 56] (Fig. 1, Step 7).

Consequences of beta-cell dysfunction are depicted in Steps 2, 3, 10 and 11 in Fig. 1[54]. Increased basal glucagon production results in increased basal hepatic glucose production[56]. Reduced incretin effect and reduced amylin levels leads to postprandial hyperglycaemia whereas an upregulation of SGLT-2 in the kidneys contributes by increased renal reabsorption of glucose. The net effect is accelerated fasting- and postprandial hyperglycaemia[54]. The action of toxic metabolites from increased glucose and fatty acid utilization results in reduced beta-cell mass and function, as well as the development of DM related micro-and macrovascular complications. The development of DM is seen as a continuum from low risk to overt disease[53]. Thus,

elevated glucose levels may be present years before DM diagnosis, which implies unawareness of the running risk for DM related complications.

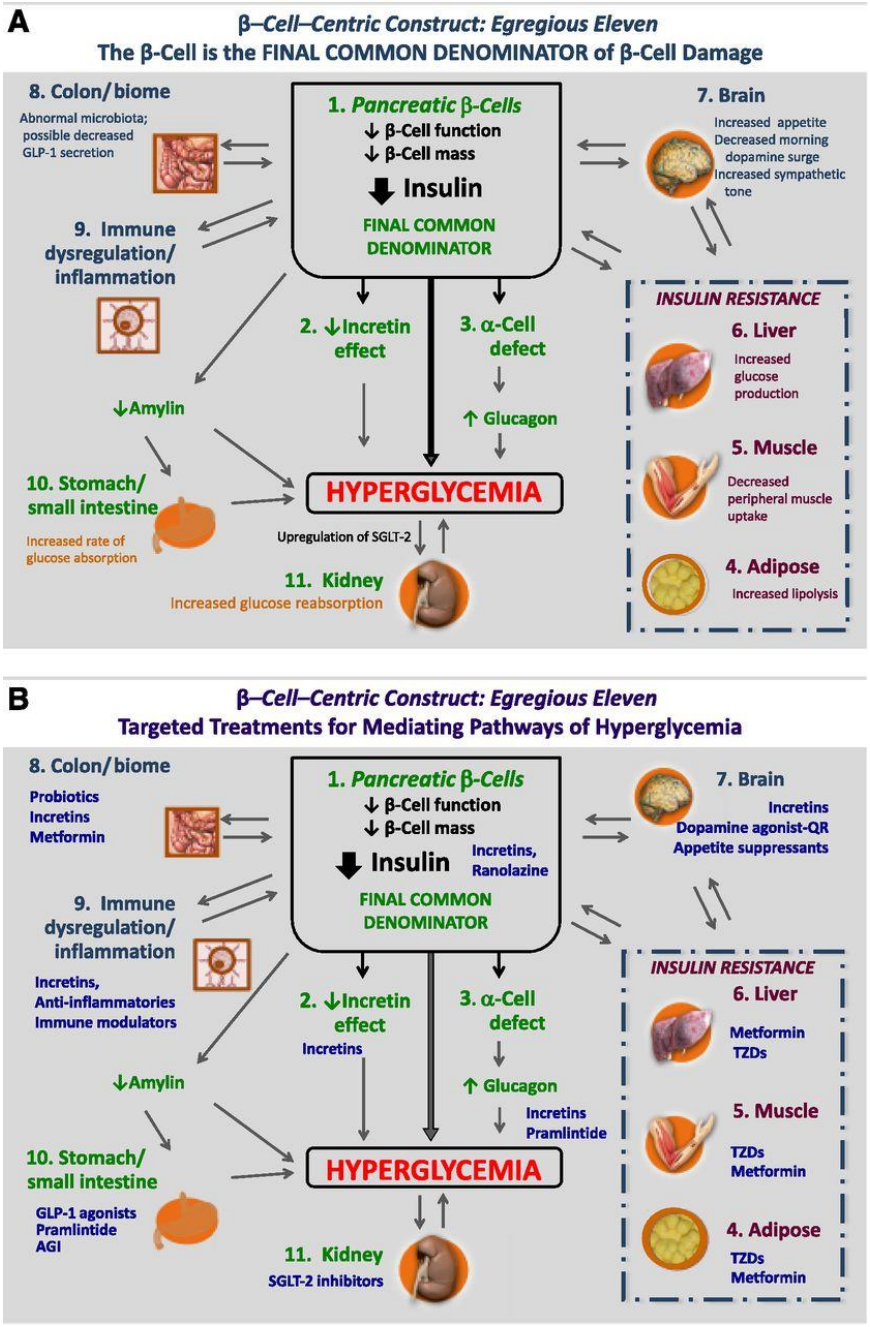

Fig. 1. A. Eleven currently known mediating pathways of hyperglycaemia. B. Current targeted therapies for each of the current mediating pathways of hyperglycemia. ©2016 by the American Diabetes Association ®. Diabetes Care 2016 Feb; 39(2): 179-18. Reprinted with permission from the American Diabetes Association ®.

1.3.2 Definition of Diabetes and Intermediate Hyperglycaemia

The definition of DM and intermediate hyperglycaemia (pre-DM)[57] is based on either glycosylated haemoglobin (HbA_{1c}) levels, fasting plasma glucose (FPG) results or a 2-hour post glucose load value following an oral glucose tolerance test (OGTT)[53, 58].

Table 1. WHO criteria for the diagnosis of diabetes and intermediate hyperglycaemia.

Diagnostic criterion*	**Diabetes mellitus**	**Intermediate hyperglycaemia**
HbA_{1c}**	≥ 48 mmol/mol (6.5%)	42-47 mmol/mol (6.0-6.4%)
Fasting plasma glucose	≥ 7.0 mmol/L	6.1-6.9 mmol/L
2-h post glucose load value	≥ 11.1 mmol/L	7.8-11.0 mmol/l

*A confirmative test is required if the tests are used for DM screening.

** HbA_{1c} should be measured using a certified method referenced to the DCCT trial.

The American Diabetes Association (ADA) uses an HbA_{1c} range of 5.7-6.4%, a FPG-range of 5.6-6.9 mmol/L and a 2-h post glucose load value of 7.8-11.0 mmol/L to define persons at high risk of developing DM[57].

Current WHO guidelines recommend HbA_{1c} as the preferred diagnostic test for DM. Also, annual screening for DM in high-risk individuals by measuring HbA_{1c} is recommended[58]. In a recent update on classification and diagnosis of DM, the American Diabetes Association equates HbA_{1c}, FPG and the OGTT as diagnostic tests for DM[59].

1.3.3 DM Classification

DM is classified as DM Type 1, DM Type 2, gestational DM and specific types of DM due to other causes. The latter category includes the monogenic types of Maturity onset diabetes in the young (MODY), DM due to pancreatic diseases and DM due to medication or chemicals[59].

DM Type 2 accounts for approximately 90% of all DM cases. Although DM is classified into distinct categories, the pathophysiology of DM is complex, and a phenotype based overlap between categories are seen[60]. Latent autoimmune diabetes of adult (LADA) is a form of autoimmune DM, classified as DM Type 1, and characterized by slow progression of beta-cell failure and a Type 2 diabetic phenotype[61]. Also, transient autoantibodies in patients with DM Type 2 have been described in a Norwegian population[62].

In a 2016 review, Leslie et al emphasized the need for a more precise characterization of DM patients to direct diagnostics and treatment towards precision medicine[60]. Recently, Ahlquist et al suggested a new subgrouping of DM patients based on parameters for the evaluation of beta-cell function and insulin resistance, the presence or absence of autoantibodies, age and BMI[63]. The subgrouping is proposed to provide information on the severity of DM and the risk for microvascular complications. However, the suggested subgrouping needs to be validated in further studies before implementation into clinical practice.

1.4 Biomarkers of Glucose Metabolism

Although the first method to detect blood glucose was developed by the Norwegian Ivar Bang[64] in the early 20th century, it was not until the 1950's that blood glucose measurements became available in clinical medicine[65]. Following the observation of a post-meal pathological elevated blood glucose level in several individuals, the OGTT was developed as a diagnostic test for DM during the 1960's[66]. Studies on blood glucose distribution following an OGTT in Pima Indians led to standardization of the test.

Fasting and non-fasting blood glucose levels and the OGTT are standardized and at present widely used as diagnostic tests for DM. Also, blood glucose measurements play a major role in monitoring DM treatment[53].

Fasting Plasma Glucose and The Oral Glucose Tolerance Test

Venous plasma glucose is measured after eight hours overnight fasting. Following oral ingestion of 75g glucose, plasma glucose is measured two hours post glucose-load for the OGTT. To prevent lowering of glucose by red blood cells, it is recommended that blood samples are centrifuged immediately to separate plasma, or drawn in tubes containing glycolytic inhibitors until centrifuged[53].

The diagnostic cut-off values of FPG and the OGTT for DM diagnosis are selected based on the threshold for increased risk of microvascular complications i.e. retinopathy and nephropathy[16, 53].

Limitations for the use of FPG and the OGTT as diagnostic tools for DM

Glucose measurements show large biological variation, analytical variation, pre-analytic instability, and they are affected by stress-related changes in blood glucose levels[67]. To be added, the OGTT is considered time consuming, relatively labour-intensive, the reproducibility of the test is low and overnight fasting is required[53, 68].

HbA_{1c}

HbA_{1c} is formed by the Maillard reaction, a non-enzymatic binding of a glucose molecule to the N-terminal end of the haemoglobin molecule (Fig. 2)[69]. HbA_{1c} reflects the average blood glucose over the past 8-10 weeks as established through the A1C-derived Average Glucose (ADAG) study (Fig. 2)[70].

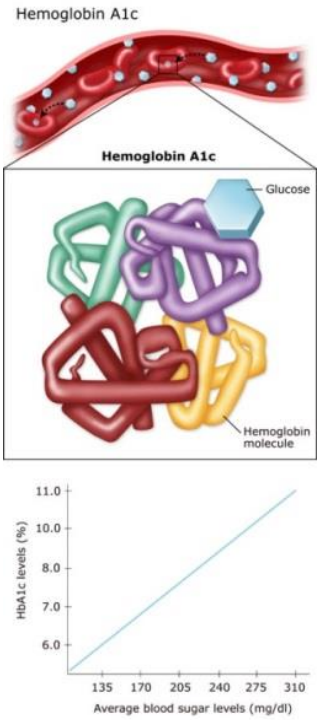

Fig. 2. The HbA_{1c} molecule and the linear relationship between HbA_{1c} and the glucose levels. The illustration is made available by written permission of The Regents of the University of California. All rights reserved.

Compared to plasma glucose, HbA_{1c} has better pre-analytic stability, less inter-day variability, is less influenced by stress, and is measured irrespective of fasting[57, 58, 71, 72]. Clinicians have used HbA_{1c} as a guidance-tool for DM treatment since the 1980's[73]. The use of HbA_{1c} in clinical practice is based on the association of HbA_{1c} with retinopathy, DM development in observational studies[71, 74, 75] and the prevention of microvascular complications by lowering HbA_{1c} level in patients with established DM[14, 17, 76]. HbA_{1c} was implemented as a diagnostic method for DM following the standardization of the HbA_{1c} assay according to a National Glycohaemoglobin Standardization Program (NGSP)-certified method referenced to the Diabetes Control and Complication Trial (DCCT)[77].

Limitations for the use of HbA_{1c} as a Diagnostic Marker for DM

HbA_{1c} measurements and results may be affected by conditions with altered erythropoiesis, erythrocyte destruction, altered glycation, variants of haemoglobin, and assay-interfering factors such as hyperbilirubinemia, hypertriglyceridemia and uremia[78]. Research has shown HbA_{1c} values to be higher with advancing age and affected by ethnicity[71, 79]. Also, HbA_{1c} is a poor discriminator for different categories of glucose dysregulation since it does not provide information about fasting glucose levels or glycaemic variability assessed by postprandial glucose levels.

1.5 PERIPHERAL ARTERIAL DISEASE – Pathophysiology, Definition and Classification

1.5.1 Pathophysiology with a Hyperglycaemic Focus

Atherosclerosis is the underlying pathological process in arterial diseases and refers to a chronic inflammatory process linked to endothelial dysfunction[80, 81]. Alterations in the interaction between the endothelium, the hemodynamics of arterial flow and blood composition initiate and facilitate atherosclerosis by inducing endothelial dysfunction[80]. DM affects all those interactions, and the risk of arterial disease in DM patients is related to the duration of DM and the presence of neuropathy[7].

DM leads to dyslipidemia and promotes atherosclerosis by inducing endothelial dysfunction due to hyperglycaemia, dyslipidemia and insulin resistance[82,83]. Also, DM is described to be a hyper-coagulable state due to enhanced platelet aggregation, increased production of tissue factor and coagulation factors, and decreased levels of antithrombotic factors[7].

During the last two decades, major research has focused on the role of endothelial dysfunction and inflammation in the development of atherosclerosis. Hyperglycaemia promotes non-enzymatic glycation of lipoproteins which affects vascular smooth muscle cell migration and apoptosis, a process of major interest for atherogenesis[80].

Glucose transporters are required for the glucose to enter the cells. Endothelial cells mostly express insulin-independent glucose transporters on their surface[84]. Hence, increased plasma glucose level will cause an increase in intracellular glucose concentration. Intracellular hyperglycaemia leads to mitochondrial overproduction of reactive oxygen species (ROS) and activation of four alternative pathways of glucose utilization; 1) increased polyol pathway flux, 2) activation of protein kinase C, 3) increased hexosamine pathway flux and 4) formation of advanced glycation end products (AGE)[85]. Together, these pathways promote atherogenesis by decreasing nitric oxide within endothelial cells, by activating several proinflammatory signals, by increasing vascular tone and vascular smooth muscle cell growth, and by

promoting thrombosis due to platelet activation and increased production of prothrombotic factors[83].

Hyperglycaemia and insulin resistance lead to the release of free fatty acids (FFA) from adipocytes. Increased mitochondrial oxidation of FFA in endothelial cells occurs following the increased level of FFA. This leads to mitochondrial ROS overproduction which in turn activate the four alternative pathways as described for hyperglycaemia with subsequent activation of proinflammatory signals[85].

The net effect of the above-mentioned pathological processes is accelerated atherosclerosis.

1.5.2 Classification and Definition of Peripheral Arterial Disease

Arterial disease is the clinical manifestation of atherosclerosis[6]. Peripheral arterial disease refers to non-coronary and extra-cranial vascular disease[86, 87]. The classification of peripheral arterial disease is based on anatomical localization and symptoms/clinical severity[87].

Classification Based on Anatomical Localization

According to current guidelines, peripheral arterial diseases are classified as carotid stenosis, abdominal aortic aneurysmal disease and/or peripheral occlusive disease. Peripheral occlusive disease includes renal arterial disease, mesenteric arterial disease, inguinal occlusive disease, and infrainguinal occlusive disease[86, 87]. In the 2017 European Society of Cardiology (ESC) guidelines, peripheral occlusive disease is named lower extremity arterial disease (LEAD)[86].

Schemes for anatomical classification of lower extremity occlusive disease are published by The Trans-Atlantic Inter-Society Consensus (TASC II) joint guidelines for the management of PAD[4]. Classification by TASC II is suggested to be used as guidance schemes for the choice of surgical treatment. Since multiple lesions often are present in advanced peripheral occlusive disease, a limitation of TASC II classification is the focus on individual lesions.

Symptom-based Classification of PAD

Several systems for the symptom-based classification of PAD have been compiled[88]. According to the 2016 Updated Guidelines of the Society for Vascular Surgery, Rutherford classification system for grading the severity of PAD is the recommended system to be used[89]. That classification is based on clinical description and objective measures of arterial circulation.

Also, a standardized grading-system for patient comorbidities is included in the current PAD guidelines[89]. The system is based on categorical grading of comorbidities and risk-factors such as diabetes, renal status, tobacco use, hyperlipidemia, hypertension, cardiac status, pulmonary status and functional status.

Definition of Carotid Stenosis and AAA

The diagnosis of carotid stenosis and AAA is based on ultrasonic measurements of the arterial occlusion or the aneurysmal expansion. AAA is defined as an aortic diameter >30mm[87]. Timing of surgical intervention is based on clinical presentation and the severity of stenosis or aneurysmal expansion[89-91].

Definition of Peripheral Occlusive Disease

An Ankel-Brachial-Index (ABI) of ≤0.90 at rest and/or post-exercise is recommended as the first test to be used for screening and diagnosis of peripheral occlusive disease[86, 92]. Due to media sclerosis, patients with DM and patients at high age may have non-compressible arteries and thereby a falsely high ABI. Toe-pressure measurement is an alternative diagnostic method in cases of incompressible arteries[86, 89, 92].

1.6 Arterial Disease and DM – Double Trouble

Although DM is one of the major risk factors for PAD, undiagnosed dysglycaemia is common in patients with peripheral arterial disease[93, 94, 45, 87]. DM prevalence in patients with PAD is four times higher than in general populations at corresponding age groups[93, 95-97]. The increased risk for PAD in persons with DM is independent of other risk factors and related to the duration of DM[44, 98].

A major challenge in clinical handling of DM is the gradual onset and delayed diagnosis. Approximately 1/3 of patients with Type 2 DM are undiagnosed and therefore untreated and at high risk of complications and increased disease burden[97, 99-101]. To facilitate early DM diagnosis, HbA_{1c} was implemented as an additional diagnostic tool for DM [58, 71, 102]. However, epidemiological studies have shown that FPG, the OGTT and HbA_{1c} largely classify different patients as having DM and pre-DM[103-107].

When using FPG and 2-h post glucose load values as criteria for the DM diagnosis, a negative association of abdominal aortic aneurysms (AAA) with DM was shown[108-110]. However, studies that aimed to compare long-term survival in AAA patients with and without DM defined by an OGTT have reported inconsistent results[110, 111].

Patients with PAD have high mortality in both a short-term and a long-term perspective[112, 113]. A two-fold excess risk of mortality in patients with PAD and DM is reported[44]. Also, patients with PAD are likely to have concomitant coronary artery disease, cerebrovascular disease and impaired renal function[114, 115]. The presence of poly-vascular disease is linked to a worse long-term outcome[116]. The combination of poly-vascular disease and DM further enhances that risk[117].

Also, persons with DM have more often advanced occlusive disease, especially below the knee, compared with non-diabetic persons[44]. Hence, patients with arterial disease and DM run a five- to ten times higher risk for major amputation and a two-fold higher risk of mortality in a four-year perspective than patients with arterial disease only[4, 44].

The main contributor of fatal health loss (death and years of life lost) in DM patients and in patients with PAD is coronary artery disease[44, 113, 118]. DM implies a double CVD risk on average[119].

Together with hyperglycaemia, additional modifiable risk factors such as hypertension, hyperlipidemia, high BMI and smoking are main contributors to the disease burden related to DM and vascular diseases[51, 120, 121]. Increasing risk for PAD for each additional risk factor is reported[122]. Hence, the risk of DM-related complications is not restricted to hyperglycaemia alone. Numerous studies on DM patients have shown that blood-pressure control, lipid lowering treatment and smoking cessation trump glucose-lowering treatment for the prevention of CVD events[123, 124].

Current guidelines on management of DM and of PAD focus on multi-target therapy aiming towards risk factor reduction in addition to glucose lowering treatment[59, 86, 87]. In addition, differences in cardio metabolic risk in persons with DM type 2 are related to the diagnostic criterion used for the diagnosis of DM[125]. This emphasises the heterogeneity of DM and the need for a systematically risk-mapping of patients in order to be able to provide tailored treatment.

In summary, extensive research has proven DM as a major risk factor for the development of vascular diseases. DM is also an additional contributor to increased disease burden in patients with PAD. For a complete risk assessment, PAD patients need to be tested for DM. At present, three different diagnostic parameters for DM are available. In this research project, the aim was to validate HbA_{1c} against FPG and 2-hour post-glucose load measurements for DM diagnosis in PAD patients. Further, information on long-term outcome following DM testing is required to evaluate the importance of a pathological test result. Therefore, based on HbA_{1c} levels, FPG values and 2-hour post-glucose load measurements, this study aimed to investigate the association of pathological test results with long-term mortality in patients with advanced PAD.

2. Aims of the Study

The overall aims in this project:

1. Evaluate the performance of HbA_{1c} as a diagnostic test for DM in vascular surgery patients with unknown glycaemic status.

2. Determine the ability of $HbA_{1c,}$ FPG and the oral glucose tolerance test (OGTT) to predict all-cause mortality during long-term follow-up.

Specific Aims:

Paper 1 To validate HbA_{1c} as a method to diagnose DM in vascular surgery patients when using the OGTT as the diagnostic gold standard for DM.

Paper 2 To investigate the prevalence of dysglycaemia in patients with abdominal aortic aneurysm and to evaluate mortality with respect to glycaemic status assessed by HbA_{1c} and OGTT measurements.

Paper 3 To evaluate HbA_{1c} and the OGTT as predictors for long-term mortality in vascular surgery patients with unknown glycaemic status.

Throughout this thesis, the term OGTT refers to the measurements of both FPG and 2-hour post glucose load values. The term PAD includes carotid stenosis, iliac occlusive disease (IOD), infrainguinal occlusive disease and abdominal aortic aneurysmal disease (AAA).

3. Materials and Methods

3.1 Study Population

This research project was a prospective cohort study of vascular surgery patients with advanced vascular disease. The vascular pathologies were carotid stenosis, iliac occlusive disease (IOD), infrainguinal occlusive disease and abdominal aortic aneurysmal disease (AAA).

Between October 2006 and September 2007, 465 patients admitted to the vascular surgery unit at Haukeland University Hospital, Bergen, Norway for elective surgery or contrast angiography, were invited to participate in the study. Of those, 66 (14%) declined to perform an OGTT and 21 (5%) died before deciding whether to participate. In addition, 33 (7%) patients did not have an OGTT performed due to logistic reasons and three patients had missing HbA_{1c} values. Sixty-seven (14%) patients had known DM.

All vascular surgery patients admitted to Haukeland University Hospital, Bergen, Norway, for treatment are included in the Norwegian Cardiovascular Disease Registry (NORKAR), governed by The Norwegian Institute of Public Health.

The study cohort in Paper I and Paper III

Patients having known DM at baseline, arterial disease of other causes than atherosclerosis and missing HbA_{1c} or OGTT results were excluded.

The study-cohort in Paper I included 275 vascular surgery patients of unknown glycaemic status, two of which with non-atherosclerotic disease. Those two patients were excluded in Paper III. Hence, in Paper III, data from 273 patients were used.

The study cohort in Paper II

Data from 66 AAA patients selected from the total cohort of vascular surgery patients was used. Patients with other vascular pathologies than AAA were excluded. Seven patients had known DM. One patient had missing HbA_{1c} result. OGTT and HbA_{1c} results from 58 AAA patients of unknown glycaemic status were included for the mortality analyses.

3.2 Baseline Data

Relevant covariates at baseline were retrieved from the patients' medical records and from data collected at the inclusion in the study. Information about the obtained patient characteristics at baseline is described in the respective papers.

Characterization of the vascular pathologies were based on findings from duplex ultrasound imaging, computed tomography angiography (CTA) with injection of iodinated contrast and, if indicated, contrast angiography. Trained ultrasound technicians at the vascular unit performed the duplex ultrasound imaging. The CTAs were obtained by radiologic technologists. Specialists in interventional radiology performed the contrast angiographies. All cases, with accompanying images, were evaluated by specialists in vascular surgery and interventional radiology in joint vascular meetings.

Based on the conclusions from the vascular meetings, patients in this research project were initially classified according to the relevant vascular pathology at inclusion.

For the mortality analyses in Paper III, the candidate performed a re-categorization of patients with iliac occlusive disease and infra-inguinal occlusive disease based on a re-evaluation of angiogram descriptions. In cases of a multiple level disease, the patients were classified according to the distal findings.

3.3 Diagnostic Tests

Glucose levels of the OGTTs and HbA_{1c} values were measured at baseline. Paper I holds a detailed description of diagnostic tests and laboratory methods.

Oral Glucose Tolerance Tests (OGTTs)

The OGTTs were performed by oral administration of 75g anhydrous glucose dissolved in water following a minimum of eight hours overnight fasting[126]. Plasma glucose was measured in a fasting state and again two hours after the ingestion of glucose. No dietary restrictions were given prior to the pre-test fasting.

In 78% of the cases, the OGTTs were performed at Haukeland University Hospital, Bergen, Norway, by a designated laboratory technician. In 61 patients (22%), the OGTT was performed at their respective General Practitioner's (GP's) offices due to logistic reasons. Of the OGTTs performed at the GP's offices, 17 (28%) were analysed at Haukeland University Hospital and 27 (44%) were analysed immediately in capillary whole blood at the respective GP's offices. Finally, plasma glucose for 17 (28%) of the OGTTs performed at the GP's offices were analysed at other regional hospitals.

Venous whole blood, drawn in containers with glycolytic inhibitors (citrate and fluoride) and centrifuged within one hour from venous sampling to separate plasma, was used for the OGTT glucose measurements performed at Haukeland University Hospital. The pre analytical handling of the blood samples for the OGTTs performed at other regional hospitals was not known.

The OGTT plasma glucose levels were analysed using the available resources at the different hospital laboratories.

In 84% of the OGTTs, the glucose measurements were performed at Haukeland University Hospital by using the hexokinase assay on Roche Modular P (Roche Diagnostics, Indianapolis, U.S.A.).

Glucose measurements for the remaining 16% of the OGTTs were performed by using the following equipment: Ortho-Clinical Diagnostics Vitros 950 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York, U.S.A.), Architect ci 8200 Integrated System (Abbott Core Laboratories, Abbott Park, Illionis, U.S.A), Architect c 8000 Clinical Chemistry Analyzer (Abbott Core Laboratories), and Roche Hitachi 911 (Roche Diagnostics).

At the GP's offices, the following resources were used for analysis: HemoCue Glucose 201+ System (Wilburn Medical Inc, Kernersville, U.S.A.), HemoCue AB B

Glucose Photometer (HemoCue Ltd. Ängelholm, Sweden) and Roche Reflotron Plus system (Roche Diagnostics, GmbH Germany).

HbA_{1c}

HbA_{1c} values were measured on all participants through a single blood sample. In 98% of the cases, HbA_{1c} was analysed in the Department of Laboratory Medicine and Pathology, Haukeland University Hospital, Bergen, Norway, using HPLC Variant II Hemoglobin testing system (Bio-Rad Laboratories, Munich, Germany). The method correlates to the DCCT reference study and is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine reference method.

Four patients were tested at their GP's office, and three patients at other regional hospitals. HbA_{1c} values were then analysed using the following resources: HPLC Variant II Hemoglobin testing system (Bio-Rad Laboratories, Munich, Germany), DCA 2000 (Siemens/Bayer Diagnostics Europe, Dublin, Ireland), DCA Vantage Analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, New York, U.S.A), Roche Hitachi 912 (Roche Diagnostics), D-10 Hemoglobin Testing System (Bio-Rad Laboratories), NycoCard Reader Axis-Shield HbA_{1c} assay (Abbott Laboratories) and Architect ci 8200 Integrated System (Abbott Core Laboratory).

External Quality Assessment of the Laboratory Methods

All performing laboratories in this research project participated in external quality assessment programs recommended or performed by the Norwegian Quality Improvement of Laboratory Examinations (Noklus).

The range of Coefficient of Variation (CV) of the methods used for glucose measurements at the different hospital laboratories were 1.8% -3.0%. The results from external quality assessment of the equipment used for glucose measurements at the GP's offices were referred to as accepted or not accepted. All reported values were accepted.

The CVs of the methods used for HbA_{1c} analyses were 0.8%-2.6% at HbA_{1c} level 5.4%-9.8%. Two blood samples were analysed on DCA 2000. CV of the DCA 2000 was 4.2%-5.2% at an HbA_{1c} level of 5.0%. All methods used for HbA_{1c} analyses correlates to the Diabetes Control and Complication Trial reference study.

Classification of glycaemic status

Based on the FPG levels, 2-hour post glucose load values and HbA_{1c} results, the patients were categorized as having normoglycaemia, intermediate hyperglycaemia (pre-DM) and DM according to ADA and WHO criteria[53, 57].

3.4 Endpoints and Follow-up

Main outcome was death of all causes. Information about deaths was obtained from the patients' medical journals, which are linked to the Norwegian civil registry. Specific causes of death were not registered.

Study participants were followed from the date of surgical intervention either to the date of death or to the date of study closure. Follow-up of the AAA patients presented in Paper II was closed on the 11th of August 2014. For the patients included in Paper III, the date of study closure was the 30th of August 2016. All study participants attended the outpatient program at the Department of Vascular Surgery at Haukeland University Hospital.

3.5 Statistical Analysis

Statistical analyses were performed in SAS, R, and Stata (Windows) and in SPSS Statistics 24 (IBM). Data were presented as mean ±standard error for continuous data, and as absolute count with percentage for categorical data. The Chi-square test was used to explore associations between categorical variables. Associations between continuous data were analysed using independent samples T-test. Inspection of histograms was used for determination of skewness and kurtosis in order to evaluate normal distribution of the variables included for analysis. Outliers, defined as diabetic extreme values of OGTT and HbA_{1c} measurements, were modified by changing their value to a less extreme value.

The association between OGTT values (FPG and 2-h post glucose load values) and HbA_{1c} values was examined by using segmented regression analysis. The performance of HbA_{1c}, when using the OGTT as the gold standard, was evaluated by

inspection of receiver operating characteristic (ROC) curves and calculations of area under the curve (AUC) (Paper I).

Cox regression models were used to estimate all-cause mortality hazard ratio with 95% confidence intervals (CIs) for HbA_{1c}, FPG and 2-h post glucose load results (Paper II and Paper III). Three adjustment models were constructed to evaluate the impact of possible confounding. Selection of covariates in the models was based on known risk factors associated with DM and PAD and established medical treatment for PAD. The proportional-hazards assumption was fulfilled for all variables in the final regression models. Survival was pictured using Kaplan Meyer Survival Curves.

3.6 Ethical Aspects

The research was carried out in accordance with the Declaration of Helsinki and principles of Good Clinical Practice. The research protocol was approved by the Regional Committee for Medical Research Ethics (REK vest 14109). Informed written consent was obtained from all participants.

4. Summary of Results

A detailed presentation of results with accompanying figures is available in the respective papers.

4.1 Paper I - Validation of HbA1c as a method to diagnose DM and intermediate hyperglycaemia in vascular surgery patients when using OGTT as the gold standard.

In the 275 participating vascular surgery patients of unknown glycaemic status, glucose levels of the OGTTs and HbA_{1c} values were measured at baseline. The patients were categorized according to WHO and ADA definition of DM and intermediate hyperglycaemia. The performance of HbA_{1c} was evaluated by AUC calculations using the OGTT as the gold standard.

Based on OGTT results, DM prevalence was 12% and the prevalence of intermediate hyperglycaemia was 33%. An HbA_{1c} value of ≥48 mmol/mol (6.5%) detected DM with 45.5% sensitivity and 90% specificity. According to HbA_{1c} results, the prevalence of intermediate hyperglycaemia was 70%. The total prevalence of DM and intermediate hyperglycaemia was 85% based on HbA_{1c} values and 45% based on the OGTTs. AUC for the association between HbA_{1c} and the OGTT as diagnostic parameters for DM was 0.73 (95% CI 0.63, 0.84).

Patients diagnosed with intermediate hyperglycaemia by OGTT results had the highest prevalence of reduced renal function and were more likely to be former or current smokers when compared to DM patients.

The conclusion reached through this study was that HbA_{1c} levels and OGTT results largely categorized different individuals as having DM and pre-DM. The total prevalence of DM and pre-DM was higher based on HbA_{1c} values than based on OGTT results.

4.2 Paper II – Evaluation of DM prevalence and mortality with respect to glycaemic status in patients with abdominal aortic aneurysms.

In Paper II, the aim was to investigate the prevalence of DM and intermediate hyperglycaemia in AAA patients and to evaluate mortality with respect to glycaemic status assessed by HbA_{1c} and OGTT measurements. Survival analyses were performed by using multivariate Cox-regression models.

The total prevalence of known and newly diagnosed DM in this study population was 23% based on OGTT results. Seven of the 66 AAA patients had known DM at baseline. The prevalence of newly diagnosed DM in 58 AAA patients of unknown glycaemic status at baseline was 12% (OGTT results) and 14% (HbA_{1c} results) respectively. Compared with OGTT results, HbA_{1c} values classified DM patients with 43% sensitivity and 90% specificity. The prevalence of intermediate hyperglycaemia (pre-DM) was 43% based on OGTT results and 72% based on HbA_{1c} values.

Mean follow-up time was 71 months [0-100] (5.9 years). Total mortality in the 58 AAA patients without known DM at baseline was 43%.

HbA_{1c} was a significant independent predictor for mortality in the DM category, adjusted HR 6.35, (95% CI 1.49-27.1); p=0.01.

Compared with patients alive at study closure, patients who died during follow-up were older, had higher prevalence of cerebrovascular disease at baseline and were more likely to be treated with an endovascular procedure. Patients who received antiplatelet therapy at baseline had significantly higher total survival than those not treated.

We concluded that AAA patients had high DM prevalence and half the patients were unaware of their DM diagnosis. DM defined by $HbA_{1c} \geq 48$ mmol/mol (6.5%) is associated with a lower survival-rate following surgical treatment for AAA. HbA_{1c} may thus be important in pre-operative risk assessment of AAA patients.

4.3 Paper III – Evaluation of HbA_{1c} and the OGTT as predictors for long-term mortality in vascular surgery patients of unknown glycaemic status.

The main objective of Paper III was to evaluate HbA_{1c}, FPG and 2-hour-post glucose load value as predictors for long-term mortality in 273 vascular surgery patients of unknown glycaemic status. Cox regression models were used to explore the associations of HbA_{1c} and OGTT glycaemic categories with all-cause mortality.

Mean follow-up time was 2805 days [0-3779] (9.2 years). All-cause mortality in the study population was 40%, and higher for AAA patients (55%) and patients with infra-inguinal occlusive disease (45%) than for those with carotid stenosis (24%) and IOD (15%).

Compared with patients being alive at study closure, patients who died during follow-up were older, had higher cardiovascular comorbidity and were less likely to use anti-platelet therapy at baseline compared with patients being alive at study closure.

HR of all-cause mortality according to HbA_{1c} values was 1.54, (95% CI 1.03 – 2.32); p=0.04 when adjusted for age, sex and the use of platelet inhibitors, statins and antihypertensive medication. The OGTT was not an independent predictor for mortality.

The results of this exploratory study suggest that HbA_{1c} is a useful risk marker in preoperative screening of PAD patients of unknown glycaemic status at the time of admission for vascular surgery. Further research is needed to confirm these results and to evaluate whether or not the results are replicable for each of the vascular pathologies.

5. Discussion

5.1 Methodological Considerations

This prospective cohort study of vascular surgery patients evaluated the performance of HbA_{1c} for diagnosis of DM when using the OGTT (FPG and 2-h post glucose load value) as a gold standard. Further, the associations between HbA_{1c}, FPG and 2-hour post glucose load values, and all-cause mortality during follow-up were assessed.

5.1.1 Study Population

In this research project, patients with advanced atherosclerotic disease and unknown glycaemic status were studied. The vascular pathologies were carotid stenosis, AAA and peripheral occlusive disease including IOD and infrainguinal occlusive disease.

Gender distributions and the presence of risk factors and vascular comorbidities in our study population were consistent with findings from other studies on vascular surgery patients[86, 108, 122, 127-129]. The authors therefore believe that the results from this study regarding diagnosis of DM are applicable on vascular surgery patients with advanced disease. The results are not necessarily applicable on patients with a milder form of vascular disease. Since the development of DM is a continuum from low risk to overt DM[53], the high prevalence of DM and pre-DM based on HbA_{1c} results may indicate a high degree of dysglycaemia even in patients with less advanced vascular disease. This implies a possibility that the results from this study-population may be relevant for vascular surgery patients with less advanced vascular disease.

Former studies of PAD patients include patients having various mixtures of glucometabolic states; DM, pre-DM, normoglycaemia and mixed populations. In this research project, patients of unknown glycaemic status were all tested using HbA_{1c}, FPG and OGTT measurements and included in the survival analyses. Hence, no patients were pre-selected based on glycaemic status.

5.1.2 Non-attendance

A high number of non-attendants may have introduced a selection bias to this research project. Selection bias due to non-response or non-attendance may contribute to a bias of estimates if the non-attendants differ from the study participants[130]. Based on information from the Norwegian Cardiovascular Disease Registry, an analysis of the non-attendants was performed. Evaluation of mean FPG level, age, gender and vascular pathology showed that patients who died before deciding whether to participate were older (median age 82.0 years) and more likely to have infrainguinal occlusive disease compared with the study population. In comparison to the study participants, a higher percentage of patients who were excluded from the study due to missing OGTTs had inguinal occlusive disease and a higher percentage of patients who declined to participate were female. Mean FPG at baseline was the same for participants and non-participants.

The fact that 21 patients died before deciding whether to participate may reflect too wide inclusion-criteria. The 21 patients had reached average life expectancy in Norway and were therefore not necessarily representative for the population in this study or vascular surgery patients in general[36]. If included in the study, the patients would represent only six percent of the study population. A selection bias regarding patients with infra-inguinal occlusive disease is believed to have only minor influence on results although it cannot be excluded.

Mortality in the 33 patients who were excluded from this study due to missing OGTT values was 55% at study closure on the 30th of August 2016. This was higher compared with 40% mortality in the study population. Of the 33 patients with missing OGTTs, 15 patients were alive at study closure. Ninety-three percent of those patients had normal FPG level at baseline compared to 78% of the patients with missing OGTTs who were dead at study closure.

In contrast, 61% of the patients with missing OGTTs who died had normal HbA_{1c} level at baseline compared to 27% of the 15 patients who were alive at study closure. Half of the patients having normal FPG levels had pre-DM according to HbA_{1c} values. Only one patient fulfilled DM criteria with $HbA_{1c} \geq 6.5\%$. Hence, the

association of glycaemic status with mortality may have been underestimated in patients with IOD since patients with missing OGTTs were more likely to have inguinal occlusive disease.

5.1.3 Classification of Vascular Pathologies

Long-term survival rate varies according to affected vascular bed[87]. Mortality in patients with carotid stenosis is reported to be 10-20% in a 5-year time period[127, 131] whilst for AAA patients it is 40-50%[132, 133]. Mortality in patients with peripheral occlusive disease is 40-50% in five years[113, 134-136]. A study from Finland revealed a higher mortality in patients with crural occlusive disease as compared with proximal occlusive disease classified according to TASC II[137]. Hence, misclassification of patients may have impact on results of survival analyses. Further, a unified classification of the vascular pathologies according to joint guidelines would make research results comparable across studies.

In the present research project, the authors considered to classify patients with peripheral occlusive disease by affected arterial segments according to TASC II. The authors also discussed to classifying those patients by symptoms according to Rutherford[88]. However, since such sub-classification was not accounted for prior to inclusion of patients in the study, the study did not have statistical power to perform analysis on the suggested categories.

5.1.4 Diagnostic Tests

When screening a population for a disease, high specificity and negative predictive value are preferable properties for the diagnostic test used. Specificity is defined as true negatives divided by true negatives plus false positives. Negative predictive value is defined as true negatives divided by true negatives + false negatives[130]. In concordance with other studies, results from this study found that HbA_{1c} level had high specificity (90%) and high negative predictive value (92%) when using the OGTT as the gold standard. As recommended by the WHO, confirmative testing should be performed following a positive test result from the initial screening test used for the diagnosis of DM[58]. Only single measurements of HbA_{1c}, FPG and 2-hour

post glucose load values were used in this research project. The FPG and 2-hour post glucose load values are known to have moderate reproducibility after retesting[53, 68]. Only half of those having pre-DM according to first FPG or 2-hour post glucose load value had pre-DM following repeated testing[68]. The majority of patients that were reclassified had normal FPG level or 2-hour post-glucose load value. Single measurements may have affected the categorization of patients into different glycaemic categories in our study. However, large epidemiological studies have used results from single measurements, thus making the results from this study comparable to other studies.

The step from a diagnostic test result until diagnostic decision-making also involves considerations regarding analytical information about the test used. Standardization of the HbA_{1c} assay has been essential to the implementation of HbA_{1c} as a diagnostic test for DM. The HbA_{1c} assays used in this study were standardized according to a National Glycohaemoglobin Standardization Program (NGSP)-certified method referenced to the DCCT trial. Pre-analytical handling and analysis of the plasma glucose tests were largely performed according to WHO recommendations[53].

When the diagnosis of DM is established, further classification into different types of DM is based on measurements of auto-antibodies and evaluation of phenotype. Auto-antibodies are used to differentiate between DM Type 2 and latent autoimmune diabetes of adult (LADA). DM related auto-antibodies were not measured in this research project. Hence, we could not differentiate between DM type 2 and LADA. However, the presence of autoantibodies is associated with a more rapid decline in beta-cell function and mass and increased need for early intensive treatment compared to having negative auto-antibodies[53, 57]. Since only nine of the 33 patients with newly diagnosed DM by OGTT results received anti-diabetic therapy at the end of the study, it is most likely that patients in this study had DM Type 2 although the possibility of patients having LADA cannot be excluded.

5.2 Discussion of Results

It is important to emphasise that this research project was not designed to explore causality for the association of glycaemic status and all-cause mortality, nor for the observed discordance in classification of patients into glycaemic categories according to HbA_{1c} and OGTT results. Hence, the presented discussion and suggested explanations are literature-based speculations.

5.2.1 Differences in Classification into Glycaemic Categories

Changes in diagnostic methods and criteria over time affect DM prevalence and incidence. The first diagnostic approach to DM was based on the sweet taste of urine[10]. Urine testing was gradually replaced by blood testing through the 20th century[64] and diagnostic limits for the diagnosis of DM were eventually introduced based on quantifying blood glucose in a fasting state and post glucose challenge[138]. Following the implementation of blood glucose testing through the 20th century, the diagnostic cut offs for DM have been debated and revised several times. The use of HbA_{1c} for the diagnosis of DM was added to the debate in the late 1990's[139]. However, lack of standardization of the HbA_{1c} assay postponed the implementation of HbA_{1c} as a diagnostic criterion until 2009[71]. HbA_{1c} was implemented to improve and facilitate the diagnosis of DM since glucose measurements have high variability, and the OGTT has low reproducibility and is inconvenient to perform[58, 68]. HbA_{1c} has most of the preferred requirements for a diagnostic test. HbA_{1c} is easily available, has low costs, is easy to use, has the ability to provide reliable results with high specificity, and has the ability to provide reliable prognostic information[58, 140].

Research has shown that using HbA_{1c} criteria for the diagnosis of DM results in a lower prevalence of DM in general populations and populations at risk of developing DM compared to the OGTT criteria[96, 103-106]. According to the results from this research project, using HbA_{1c} levels gave the highest prevalence of DM and pre-DM compared to when using OGTT criteria. This is in concordance with the findings in a multi-ethnic cohort, in a Chinese population and from the Tromsø OGTT study[141-143]. However, the participants in the Tromsø OGTT study were preselected based on

HbA_{1c} values, which may have led to skewed estimations. On the contrary, Doerr et al found a lower prevalence of newly detected diabetes in CAD patients when using HbA_{1c} criteria compared to when using OGTT criteria[105]. This was confirmed in the Euroaspire IV study by screening CAD patients for DM using both HbA_{1c} and OGTT measurements. Ninety-six percent of patients with newly diagnosed DM were identified by OGTT results whereas HbA_{1c} detected 17%[144]. Hence, implementing HbA_{1c} as a diagnostic test for the diagnosis of DM has had inconsistent impact on DM prevalence in different populations.

Particularly high is the number of patients categorized as having pre-DM according to HbA_{1c} results as compared with OGTT results in this research project. In Paper I, an HbA_{1c} range of 5.7-6.4% for the diagnosis of pre-DM was used. This was in accordance with ADA's criteria for the diagnosis of pre-DM. However, in 2011 the WHO implemented an HbA_{1c} range of 6.0-6.4% for pre-DM[58]. Using WHO criteria, 121 (45%) patients had pre-DM according to HbA_{1c} results and 111 (41%) had normal HbA_{1c} (Paper III, Table 1). The corresponding number of pre-DM patients was 193 (70%) and that of normal HbA_{1c} was 42 (15%) when using ADA criteria (Paper I, Table 3). The OGTT results defined 90 (34%) patients as having pre-DM. Normo-glycaemia was found in 55% according to OGTT results. In conclusion, the prevalence of pre-DM in patients having advanced peripheral arterial disease is higher based on the by ADA and WHO proposed HbA_{1c} ranges than based on OGTT results.

Prediction of clinical consequences of a positive test-result must be added to the evaluation of the diagnostic tests used for clinical decision-making[145]. According to the 2010 ADA guidelines and the 2011 WHO statement on the use of HbA_{1c} for the diagnosis of DM, patients having pre-DM or DM of HbA_{1c} < 53 mmol/mol (7.0%) are recommended lifestyle interventions and annual monitoring of glucose status[58, 59]. According to the results from this research project, using HbA_{1c} as the diagnostic test for the diagnosis of DM will imply a substantially higher number of patients in need for a close clinical follow-up compared to when using the OGTT.

The performance of a diagnostic test may be affected by the pre-test probability in the population tested[130]. In a population with low pre-test probability regarding a specific disease, a diagnostic test of high sensitivity to detect the disease will have low positive predictive value. The prevalence of DM and pre-DM is substantially higher in patients with peripheral vascular disease compared to in general populations at corresponding age groups[45, 93, 97, 99]. Hence, the pre-test probability for DM in patients with peripheral vascular disease is high.

In general populations, pre-DM according to OGTT results is more common than pre-DM according to HbA_{1c} criteria[103, 146]. Thus, pre-test probability of pre-DM defined by OGTT results is higher than when based on HbA_{1c} levels. This may imply that the OGTT has a higher positive predictive value than HbA_{1c} when identifying pre-diabetic individuals in general populations. As opposed to the results from general populations, pre-DM in this study was substantially higher based on HbA_{1c} results than based on OGTT results. This may imply that HbA_{1c} has a higher positive predictive value when identifying pre-diabetic vascular surgery patients than the OGTT, and hence would be the preferred test to use in this group of patients. However, this assumption pre-supposes a close to equal sensitivity and specificity of HbA_{1c} and the OGTT when used as diagnostic tests.

5.2.2 Diagnostic Cut-off

To evaluate if a diagnostic test is clinically valuable, the test's discriminative ability to tell if a person has a disease or not must be considered.

Test results may have a binary or a continuous outcome. The development of DM is seen as a continuum from normoglycaemia to overt DM[53]. Hence, measurements of FPG, 2-hour post glucose value and HBA_{1c} have continuous outcomes varying from low risk of DM to overt disease. Implementation of a diagnostic cut-off for clinical decision-making may be used when a diagnosis is based on continuous variables. However, to choose a clinically useful cut-off may be challenging. The cut-offs for diagnosing DM are based on thresholds for increased risk of retinopathy although there is no definite division between a healthy stage and DM[71, 75].

In this study, HbA_{1c} had low sensitivity (45.5%) and positive predictive value (38%) for the diagnosis of DM compared with OGTT results. However, these calculations are based on a diagnostic cut off which in theory defines a person as either sick or healthy. Interpretation of a clinical cut off may be challenging. A person standing with one foot aligned with the diagnostic limit for DM and the other foot next to the first, will probably have close to equal risk of cardiovascular complications as a person standing just across the diagnostic limit.

Introduction of a high-risk group may be used to address the problems using a diagnostic cut-off for continuous measurements. A high-risk range of HbA_{1c}, FPG and 2-hour post glucose load value is established instead of a precise lower cut-off [58]. In this research project, patients were classified into different glycaemic categories according to cut-offs defined by the WHO and ADA[53, 57]. However, when exploring the association of diagnostic parameters with mortality, HbA_{1c}, FPG and 2-hour value were used as continuous variables. The use of continuous variables provides a more realistic model since the relationship of the diagnostic parameters with DM is linear. The optimal retinopathy-based cut offs for the diagnosis of DM is not consistent in different studies, and DM cut-offs are arbitrary since the development of DM is a continuum. Also, the use of continuous variables in a regression analysis provides a greater precision and power from a statistical point of view[147]. Hence, the results regarding association of HbA_{1c} and the OGTT with mortality are not affected by the use of a diagnostic cut-off.

5.2.3 The Use of a Gold Standard in Clinical Decision-making

A receiver operating characteristic (ROC) curve may be used to evaluate and picture the performance of a continuous variable for a specific diagnosis.

The Receiver Operating Characteristic curve was developed during the Second World War by plotting radar-operators' evaluations of radio signals against arriving enemy aircraft. In medicine, the ROC curve was introduced in a variety of areas to assist clinical decision-making by evaluating diagnostic tests[148].

The use of a cut off categorizing obtained data as positive and negative is the basis for calculating a ROC curve[149]. Every point in the ROC curve may be described as a trade-off between sensitivity and specificity.

However, a challenge in medicine is how to define a pathological condition. Information retrieved by the ROC curve will depend on the parameter used as the gold standard. The OGTT was used as the gold standard for the diagnosis of DM in this research project. The results of the study showed an area under the curve (AUC) of 0.73, which may be considered moderate performance of HbA_{1c} compared with the OGTT.

A problem using a gold standard is that all other tests will be inferior[150]. The use of an external parameter linked to the relevant condition could bypass the problem regarding the use of a gold standard. Diagnostic cut offs for DM are based on the presence of retinopathy[53, 58]. Therefore, the presence of retinopathy obtained by fundus photography is a possible external parameter to be used when evaluating the performance of HbA_{1c} for the diagnosis of DM in comparison with the OGTT. As stated earlier, the main contributors to the disease burden related to DM and vascular diseases are dysglycaemia, hypertension, dyslipidemia, high BMI and smoking. In a Chinese population, Peng et al found that patients diagnosed with DM solely based on $HbA_{1c} \geq 48$ mmol/mol (6.5%) had the worst cardiovascular risk profile[142]. Hence, instead of using the OGTT as a gold standard, a metabolic risk profile could be used as an external parameter when evaluating HbA_{1c} versus the OGTT for the diagnosis of DM.

5.2.4 Pathophysiological Differences between HbA_{1c} and the OGTT

The limited concordance between glycaemic categories according to HbA_{1c}, FPG and 2-h post glucose load results may to some extent be explained by differences in pathophysiological mechanisms of the tests.

The 2-h post-glucose load value following an OGTT represents a stress test of beta-cell functioning. By reflecting the average blood glucose level over the past 8-12 weeks, HbA_{1c} is a marker of chronic glycaemia.

Insulin resistance is a key feature in the pathophysiology of DM. Hepatic insulin resistance is the main contributor to fasting hyperglycaemia by elevated hepatic glucose output and impaired early insulin secretion. A pattern of insulin resistance in muscle and adipose tissue combined with impaired late phase insulin secretion is characteristic of postprandial hyperglycaemia[151]. The result is prolonged post glucose load hyperglycaemia. Patients with combined elevated FPG level and 2-hour post glucose load value have a worse metabolic profile than those patients having fasting hyperglycaemia or 2-hour hyperglycaemia only[151, 152]. Also, metabolic changes precede a diagnosis of DM by several years[125].

In the Tromsø OGTT study, patients with solely OGTT-defined pre-DM had a worse metabolic risk profile than patients having pre-DM by HbA_{1c} results only[143]. However, in a study from China, OGTT negative patients with DM by HbA_{1c} results were older, had higher prevalence of CAD, HT and obesity, and were more likely to be smokers compared with OGTT positive patients with normal HbA_{1c}[142].

Based on a healthy population, the National type 2 diabetes prevention programme in Finland (Fin-D2D)-study found that a specific HbA_{1c} level implied relatively higher 2-hour post glucose and slightly lower FPG in older individuals when compared to in younger individuals[153]. Also, studies have shown that HbA_{1c} and FPG correlates stronger for higher levels of HbA_{1c} than for lower[70].

In the study-population of this study, advanced vascular disease and a high degree of vascular comorbidities precedes the diagnosis of DM and pre-DM. Vascular inflammation is a key element in endothelial dysfunction in DM[9, 154]. Studies have described an association of HbA_{1c} with endothelial dysfunction both in persons with DM and in non-diabetic persons [154-156]. In non-diabetic individuals, an association of inflammatory markers at baseline with increase in HbA_{1c} levels during a seven years follow-up was shown[154].

To speculate, DM diagnosed using glucose criteria (fasting plasma glucose and 2-hour post glucose load results) may represent a moderate age-related DM and therefore have little impact on long-term mortality risk. In contrast, HbA_{1c} expresses long-term exposure to glucose and is linked to the degree of vascular inflammation.

HbA_{1c} may thus act as a surrogate marker for a worse metabolic profile in patients with advanced vascular disease and, therefore, be related to a higher risk of a shorter lifespan.

5.2.5 Association of HbA_{1c} and OGTT Categories with Long-term Mortality

The survival rate in vascular surgery patients is three to five times lower than that of a general population at corresponding age groups and twice lower than that of patients with advanced CAD[112, 157-160]. Patients with both arterial disease and DM have higher mortality compared to patients with arterial disease only[44]. Cardiovascular diseases are a main contributor to fatal health-loss (death and years of life lost) in people with DM as well as in people with macrovascular diseases[36, 113, 118].

Results from this research project showed that HbA_{1c} was associated with all-cause mortality both in patients with AAA and in the total study population of vascular surgery patients (Paper II and Paper III). While the results remained statistically significant for AAA patients in a multi-adjusted model, the association of HbA_{1c} with all-cause mortality in the total study-population of vascular surgery patients did not achieve statistical significance after multiple adjustments. However, the effect size remained mainly unchanged.

When evaluating results from a statistical analysis, the size of the measured effect and the precision of the estimates must be considered in addition to the p-value[161]. The p-value represents the probability of an observed value in the context of the null hypothesis. However, the size of the p-value does not necessarily imply a certain effect of the observed value. In statistical calculations, a large effect can produce a high p-value if the sample size is small. On the contrary, a small effect can produce a low p-value if the sample size is large enough.

In this study, HbA_{1c} was an independent predictor for all-cause mortality in Cox regression Model 1 and Model 2 (Paper III). Given a HR of 1.39, lack of statistical power due to an insufficient sample size could explain the high p-value in Cox regression Model 3. To be added, the 95% CI of the calculated HRs reflects an

acceptable precision of the estimates. This might indicate a preserved association of HbA_{1c} with mortality in a multivariate model. The authors therefore hypothesize that HbA_{1c} may be a useful marker to identify persons at high risk of long-term mortality following surgical treatment for peripheral arterial disease.

Professor Rydén et al. reported "abnormal glucose tolerance as an important predictor of long-term outcome after myocardial infarction"[162]. According to OGTT results in this research project, patients identified with pre-DM had higher mortality compared with normoglycaemic patients in crude analysis as pictured by the Kaplan-Meyer survival curve. Patients with DM tended to have higher survival-rate than patients with pre-DM. Compared with normoglycaemic patients, patients having intermediate hyperglycaemia by OGTT results were more likely to be former or current smokers. We did not assess smoking status at time of study closure. However, it is possible that a higher percentage of patients diagnosed with DM ceased to smoke. Therefore, an "exposure-to-risk" bias as a possible explanation for the differences in survival cannot be excluded. In concordance with results from this study, pre-DM according to preoperative OGTT in Dutch vascular surgery patients was associated with increased risk of cardiovascular events and cardiovascular death when compared to normoglycaemic patients and patients with DM[163]. However, results from a large study cohort of Chinese CAD patients shows that patients with newly diagnosed DM by OGTT results have lower survival rate compared to patients having pre-DM. Both pre-DM and DM were associated with cardiovascular mortality[164]. Results from the Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe (DECODE) study group showed that patients with pre-DM and previously unknown DM from OGTT criteria had higher 9-year mortality compared with normoglycaemic patients. Patients with DM had the highest mortality[165]. Both the OGTT and HbA_{1c} were found to be predictors of CVD mortality in a long-term follow-up, whereas only the OGTT was a predictor of all-cause mortality[166].

The results of this study and the study on Dutch CAD patients regarding higher mortality in pre-DM patients by OGTT results may be affected by an insufficient sample size.

In addition, in this study, patients with DM by OGTT results were informed about their DM diagnosis at baseline. At the time of inclusion in this study, HbA_{1c} was not implemented as a diagnostic test for DM. Therefore, the 25 patients having $HbA_{1c} \geq$ 48mmol/mol (6.5%) and a non-DM OGTT result were not diagnosed with DM (Paper I, Table 3). Hence, the differences in treatment of DM are another possible explanation for the differences in mortality. On the other hand, only nine of the patients with DM according to OGTT results received medical treatment for DM at study closure. Of those, five had also DM by HbA_{1c} results thus reducing the difference in medical treatment of DM between the groups.

Regardless of the OGTT results, the HbA_{1c} seems to be a useful marker for long-term mortality in vascular surgery patients. HbA_{1c} should therefore be included when assessing a risk profile in vascular surgery patients.

6. Conclusions

The following conclusions from this research project can be drawn based on the presented results and discussion:

- Vascular surgery patients have high prevalence of undiagnosed DM and pre-DM.

- The OGTT and HbA_{1c} largely identify different patient groups as having DM and pre-DM. The number of vascular surgery patients having DM and pre-DM is higher based on HbA_{1c} values than based on OGTT measurements.

- In AAA patients of unknown glycaemic status, DM defined by HbA_{1c} criteria is an important factor in mortality-risk assessment following surgical treatment for AAA.

- In patients with advanced vascular disease and of unknown glycaemic status, HbA_{1c} may be a useful marker to identify patients at high risk of lower survival rate following surgical treatment for the vascular disease.

7. Future Perspectives

The results of observational studies provide information on associations between the parameters of interest and not on the causality behind the observed associations. Consequently, they may be described as hypothesis generating. To confirm the results from the mortality analyses in this research project and to evaluate whether or not the results are replicable for each of the vascular pathologies, further research on the association of glycaemic status with long-term survival in vascular surgery patients is needed.
The link between the degree of chronic vascular inflammation and HbA_{1c} levels as a possible explanation for the high prevalence of DM and pre-DM according to HbA_{1c} results in patients with advanced vascular disease is an interesting aim for possible future research.

Studies on animal models have demonstrated that incretin-based treatment suppresses the progression of atherosclerosis as well as having a marked anti-oxidative and anti-inflammatory effect on endothelial cells[81, 167]. An incretin-based treatment study could provide additional information regarding HbA_{1c} and inflammation in vascular surgery patients.

In a comment to the 2007 update of TASC II, George Antoniou underlined the importance of an interdisciplinary cooperation in the management of PAD patients[168]. This research project was a cooperation between the Department of Vascular Surgery and the Department of Endocrinology Haukeland University Hostpital, Bergen,Norway. Following the results on AAA patients in this project (Paper II), a national study on AAA patients in Norway (the ABdominal ANeurysms and DIAbetes (ABANDIA) study) addressing dysglycaemia defined by HbA_{1c} levels has been initiated.

Further, Norgren et al. emphasized the importance of focusing on the assessment of patients' comorbidities and risk factors in clinical handling of PAD patients[4]. Considering the gradual onset of DM and the fact that metabolic changes precede the diagnosis of DM, it may be implied that it is equally important to investigate pre-DM.

It also underlines the importance of making effort in early disease detection, improved classification of dysglycaemic PAD patients, and to risk-map the patients in order to provide tailored treatment.

Hence, a national study on vascular surgery patients aiming to risk map the patients and facilitate DM diagnosis is most welcome.

Finally, atherosclerosis is described as the common pathological process leading to both peripheral occlusive disease as well as aneurysmal disease. Most studies on patients with AAA have described a negative association of DM with the development of AAA although no causality is proven to explain these associations. Pericytes are supportive cells with contractile properties located around the endothelial cells. Pericytes have been linked to the development of DM retinopathy[169-171]. Studies aiming to explore whether altered pericyte qualities could explain part of the pathological differences between occlusive and aneurysmal disease would be of interest.

8. Erratum

Paper I, Table 1:

The authors found errors in Table 1 after publication of the original article (Paper I). The correct values for medical history of coronary artery disease (CAD) at baseline were 110 (40%) of all patients, 55 (36.2%) of the patients were categorized as having normoglycaemia, 41 (45.6%) of the patients were categorized as having intermediate hyperglycaemia, and 14 (42.4%) of the patients were categorized as having DM. All presented numbers and calculations in Table 1 have been checked and no other errors were found. The presented errors did not affect the results, scientific content or conclusions. The published Erratum containing the corrected Table 1 is attached to this thesis.

Paper II, Fig. 1: Correction to the figure legend for Fig. 1: The Kaplan Meyer curves picture **overall** survival in patients grouped according to HbA_{1c} results (a) and OGTT results (b), and not diabetes-related related survival.

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CARDIO VASCULAR DIABETOLOGY

ORIGINAL INVESTIGATION Open Access

HbA_{1c} versus oral glucose tolerance test as a method to diagnose diabetes mellitus in vascular surgery patients

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Abstract

Background: The diagnosis of diabetes mellitus (DM) is based on either fasting plasma glucose levels or an oral glucose tolerance test (OGTT). Recently, an HbA_{1c} value of ≥ 48 mmol/mol (6.5%) has been included as an additional test to diagnose DM. The purpose of this study was to validate HbA_{1c} versus OGTT as a method to diagnose DM in vascular surgery patients.

Methods: The study population consisted of 345 patients admitted consecutively due to peripheral arterial disease. Sixty-seven patients were previously diagnosed with DM. Glucose levels of OGTT and HbA_{1c} values were analyzed in 275 patients. The OGTT results were categorized into three groups according to the World Health Organization 1999 criteria: 1) DM defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or two-hour value (2-h-value) ≥ 11.1 mmol/L; 2) intermediate hyperglycaemia, which consists of IGT (FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L), and IFG (fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h-value); and 3) normal glucose metabolism defined as FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L.

Results: Of the 275 patients on whom OGTT was performed, 33 were diagnosed with DM, 90 with intermediate hyperglycaemia and 152 had normal glucose metabolism. An HbA_{1c} value of ≥ 48 mmol/mol (6.5%) detected DM with a 45.5% sensitivity and a 90% specificity compared with the OGTT results. Combining the measurements of the HbA_{1c} value with the fasting plasma glucose level (≥7.0 mmol/L) increased the sensitivity to 64%. The total prevalence of DM and intermediate hyperglycaemia was 85% based on HbA_{1c} values and 45% based on the OGTT.

Conclusions: Compared with the OGTT the HbA_{1c} cut-off value of ≥ 48 mmol/mol (6.5%) had a 45.5% sensitivity to diagnose DM in patients with peripheral arterial disease. OGTT and HbA_{1c} categorized different individuals with DM and intermediate hyperglycaemia. The total prevalence of pathologic glucose metabolism was substantially higher based on HbA_{1c} values than based on OGTT. The high prevalence of DM and intermediate hyperglycaemia when using HbA_{1c} in this study may reflect a high chronic glycaemic burden in patients with peripheral arterial disease. Further studies on vascular surgery patients are needed to identify which method, OGTT or HbA_{1c}, is the better in predicting DM and future clinical development of vascular disease.

Trial registration: REK vest 14109

Keywords: Diabetes mellitus, HbA_1c, OGTT, Peripheral arterial disease

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Background

The diagnosis of diabetes mellitus (DM) has, until recently, been based on blood glucose levels, i.e. either fasting plasma glucose (FPG) ≥7.0 mmol/l or an oral glucose tolerance test (OGTT) result of ≥11.1 mmol/l [1].

The HbA_{1c} value reflects the average blood glucose over a 2-3 month period and has traditionally been used to evaluate the treatment of established DM. It has been described as a predictor for DM and of micro- and macrovascular disease [2-6]. Studies have shown a linear increase in retinopathy prevalence for HbA_{1c} at 48 mmol/mol (6.5%) and above [7,8]. However, an increase in retinopathy is also seen in the intermediate hyperglycaemia (prediabetes) range of HbA_{1c} and the overall risk assessment for DM is seen as a continuum from low risk to established DM [8].

The International Expert Committee of Diabetes, American Diabetes Association and the World Health Organization (WHO) have included HbA_{1c} value ≥ 48 mmol/mol (6.5%) as an additional method for diagnosing DM [8-10].

Two intermediate HbA_{1c} ranges of 39-46 mmol/mol (5.7-6.4%) (American Diabetes Association) and of 42-46 mmol/mol (6.0 – 6.4%) (the International Expert Committee) have been suggested to be used to identify individuals at high risk for developing DM [8,9]. The WHO has not yet made a statement on the HbA_{1c} diagnostic range of intermediate hyperglycaemia.

Previous studies have revealed a higher prevalence of DM in patients with peripheral arterial disease (PAD) [11-13] compared to general populations [14-16] and populations at risk of developing DM [17]. In all age groups the prevalence of DM in Norway is estimated to be 2.3%, with a prevalence of 3.4% among those aged ≥30 years. This increases with age to approximately 8% amongst the elderly (70-79 years) [18]. Results from the Nord-Trøndelag Diabetes Study indicate a prevalence of IGT in Norway at 0.9% in men and 0.2% in women using WHO 1980 criteria (IGT: 2-h-value between 8.0 mmol/L and 10.9 mmol/L). The study was based on a pre selection of patients with an abnormal non-fasting glucose value (≥ 8.0 mmol/L). The use of an initial screening test and a threshold value for follow-up at 8.0 mmol/L may have led to an underestimation of the total IGT prevalence[19]. The prevalence of impaired fasting glucose (IFG) in Norway is not known. A prior publication based on this study material revealed a prevalence of pathologic glucose metabolism of 55% and a frequency of diabetes of 29% among Norwegian vascular surgery patients [13].

Most studies that investigated the use of HbA_{1c} values against OGTT as a diagnostic tool for DM have found reduced prevalence by HbA_{1c} criteria compared with the OGTT criteria. The studies also showed discordance between OGTT and HbA_{1c} values suggesting that the two methods define different patient categories [14-17,20,21]. Patients with PAD are multimorbid and of high age[13]. It is important to evaluate whether results from studies on general populations and populations at risk of developing DM are applicable on this high-risk population of patients with PAD. No previous studies that have validated the use of HbA_{1c} values against OGTT in the diagnosis of DM in vascular surgery patients could be found, hence the purpose of the present study.

Methods

Patient selection

This study was a prospective cohort study. The study population consisted of 345 patients admitted consecutively to the vascular surgery unit for elective surgery between October 2006 and September 2007. Initially 466 patients were asked to participate, however 121 declined. DM was previously diagnosed in 67 patients (Figure 1). This left data from 275 patients (273 ethnic Norwegians and 2 white Europeans) for analyses. Informed written consent was obtained from all participants. The research

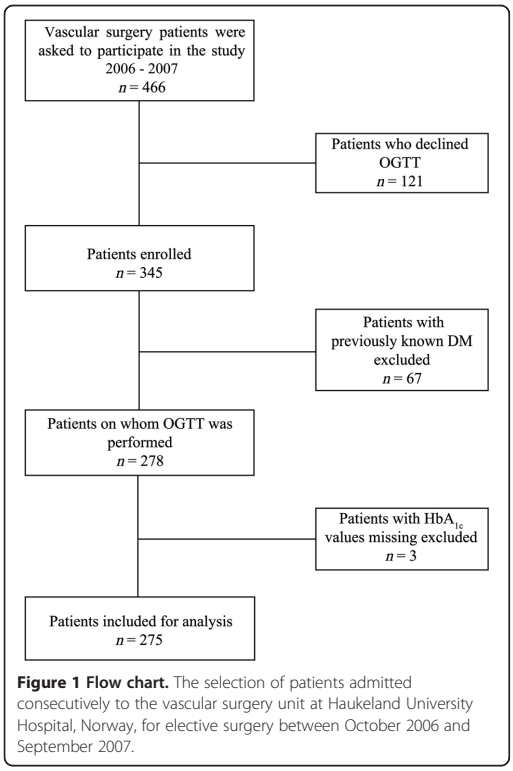

Figure 1 Flow chart. The selection of patients admitted consecutively to the vascular surgery unit at Haukeland University Hospital, Norway, for elective surgery between October 2006 and September 2007.

protocol was approved by the Regional Committee for Medical Research Ethics (REK vest 14109). The vascular pathologies were peripheral arterial disease including iliac occlusive disease (IOD), infrainguinal occlusive disease, abdominal aortic aneurismal disease and carotid stenosis.

Diagnostic tests

An OGTT was performed on the 275 participating patients. Fasting glucose and HbA_{1c} values were measured in all patients. OGTT was performed after a minimum of 8 hours overnight fasting, by orally administering a standard dose of 75 g anhydrous glucose dissolved in water. Plasma glucose levels were measured in a fasting state prior to administering the anhydrous glucose and again two hours after its administration. The patients were not recommended any special diet prior to the OGTT. In 61 patients (22%) the OGTT was performed at their respective General Practitioner's (GP's) offices due to logistic reasons. The results from these tests were also analysed at their GP's offices in 27 patients (44%), at Haukeland University Hospital in 17 patients (28%) and at other regional hospitals in 17 patients (28%). Venous whole blood, drawn in containers with glycolytic inhibitors (citrate and fluoride) and centrifuged within one hour from venous sampling to separate plasma, was used for the OGTT glucose measurements performed at Haukeland University Hospital. Blood glucose during the OGTT performed at the GP's offices was analysed immediately in capillary whole blood. The preanalytical handling of the bloodsamples for the OGTTs performed at other regional hospitals is not known.

The OGTT plasma glucose levels were analysed using the available resources at the different hospital laboratories: Modular P (Roche Diagnostics) (78% of the blood samples were analysed using Modular P), Vitros 950 (Ortho-Clinical Diagnostics), Architect ci 8200 (Abbott), Architect c 8000 (Abbott), and Hitachi 911 (Roche Diagnostics). At the GP's offices the following resources were used for analysis: Hemocue 201+ (Photometer), Hemocue B-glucose promedico and Reflotron +. The range of the coefficients of variation on the equipment used for glucose value analyses at the different hospital laboratories was 1.8% -3.0%. Analytical discrepancies on the equipment used for analysis at the GP´s offices were referred to as accepted or not accepted, and were all within accepted values set by NOKLUS.

External quality assessment of all equipment used for analysis was performed by NOKLUS. NOKLUS is a national institution certified by The National Institute of Technology (NS-EN ISO 9001:2000), and run by a committee consisting of representatives from The Norwegian Crown, The Norwegian Medical Association and The Norwegian Association of Local and Regional Authorities. NOKLUS is quality checked by The European Reference Laboratory for Glycohemoglobin in the Netherlands.

The OGTT results were categorized into three groups according to the WHO 1999 criteria: 1) DM defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/l and/or two-hour value (2-h-value) ≥ 11.1 mmol/l 2) intermediate hyperglycaemia, which consists of IGT defined as FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L, and IFG defined as fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h -value and 3) normal glucose metabolism defined as FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L.

HbA_{1c} values were measured on all participants through a single blood sample, mainly at Haukeland University Hospital (98% of the blood samples were analysed in the laboratory at Haukeland University Hospital using Variant II HPLC system). Four patients were tested at their GP's office, and three patients at other regional hospitals. HbA_{1c} values were then analysed using the following resources: Variant II HPLC system (BioRad), DCA2000, DCA Vantage (Siemens), Hitachi 912 (Roche Diagnostics), D-10 (BioRad), Nycocard reader (Axis-Shield) and Architect ci 8200 standardized immunoassay. The range of the coefficients of variation on the equipment used for HbA_{1c} level analyses was 0.8%-2.6% at HbA_{1c} values 5.4%-9.8%. Two bloodsamples were analysed on DCA 2000 with coefficients of varation of 4.2%-5.2% at HbA_{1c} 5.0%. The analysing equipment used standardised assay in accordance with DCCT standard.

The HbA_{1c} results were categorized as: DM, intermediate hyperglycaemia and normoglycaemia. The diagnostic limit of HbA_{1c} is ≥48 mmol/mol (6.5%) according to WHO statement 2011. The American Diabetes Association definition of intermediate hyperglycaemia at 39-46 mmol/mol (5.7-6.4%) was used since WHO has not yet made a statement on the HbA_{1c} diagnostic range of intermediate hyperglycaemia.

Other variables

Information about age (continuous), sex (men/women), smoking habits (yes/no), affected vascular bed, state of anaemia (yes/no) and kidney failure (yes/no) was obtained from the patients' medical records. The presence of kidney failure and anaemia was defined based on estimated glomerular filtration rates and serum haemoglobin levels. Estimated GFR values were calculated by The Modification of Diet in Renal Disease equation. According to international recommendations all patients treated at the Vascular Surgery Department were given statins and anti-platelet medication, unless strong contraindications were present. Angiotensin Converting Enzyme Inhibitor/Angiotensin receptor blocker was the preferable choice when treating hypertension.

Statistical analysis

Data were analysed using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina) and R version 2.8.1 (The R Foundation for Statistical Computing, www.r-project.org) software for Windows. All p values were 2-sided, and values <0.05 were considered statistically significant.

The data is presented as mean±standard error for continuous data and as percentage±standard error for categorical data. Correlation between pairs of continuous measures was calculated using the Spearmans correlation coefficient. Associations between categorical variables were analysed using χ^2 test. When the expected number of observations in one or more categories was ≤ 5, we used the Fisher's exact test.

Segmented regression analysis (the segmented package in R) was used to examine the association between the

Table 1 Baseline characteristics of the study population

Characteristics	OGTT				
	All patients	Normo-glycaemia	Intermediate hyperglycaemia	Diabetes mellitus	P-value[a]
Total	275	152	90	33	
Age, mean years	69.5	68.0	71.5	71.1	0.01
range]	[35-89]	[35-87]	[48-89]	[59-88]	
Sex, *n* (%)					0.02
Female	74 (26.9)	51 (33.6)	15 (16.7)	8 (24.2)	
Male	201 (73.1)	101 (66.5)	75 (83.3)	25 (75.8)	
Smoking status, *n* (%)					0.06
Non-smoker	42 (15.3)	23 (15.1)	10 (11.1)	9 (27.3)	
Former/current smoker	221 (80.4)	123 (80.9)	77 (85.6)	21 (63.6)	
Missing	12 (4.36)	6 (3.95)	3 (3.33)	3 (9.09)	
Renal function, *n* (%)					0.04
Normal (eGFR > 60)	201 (73.1)	120 (79.0)	57 (63.3)	24 (72.7)	
Reduced (eGFR < 60)	71 (25.8)	31 (20.4)	31 (34.4)	9 (27.3)	
Missing	3 (1.09)	1 (0.66)	2 (2.22)		
Anemia female, *n* (%)					
No anemia	61 (82.4)	43 (84.3)	10 (66.7)	8 (100)	0.52[b]
Female Hb < 11.7 g/dL	6 (8.11)	4 (7.84)	2 (13.3)	0	
Missing	7 (9.46)	4 (7.84)	3 (13.3)	0	
Anemia male, *n* (%)					
No anemia	163 (81.1)	81 (81.2)	62 (82.7)	19 (76.0)	0.81[b]
Male Hb < 13.4 g/dL	29 (14.4)	16 (15.8)	9 (12.0)	4 (16.0)	
Missing	9 (4.48)	3 (2.97)	4 (5.33)	2 (8.00)	
Medical history of CAD, *n* (%)					0.67
No	231 (84.0)	129 (84.9)	73 (81.1)	29 (87.9)	
Yes	42 (15.3)	22 (14.5)	16 (17.8)	4 (12.1)	
Missing	2 (0.73)	1 (0.66)	1 (1.11)	0	
Affected vascular bed, *n* (%)					0.16
Carotid	43 (15.6)	26 (17.1)	11 (12.2)	6 (18.2)	
Aortic	59 (21.5)	30 (19.7)	23 (25.6)	6 (18.2)	
IOD	50 (18.2)	35 (23.0)	9 (10.0)	6 (18.2)	
Infrainguinal	123 (44.7)	61 (40.1)	47 (52.2)	15 (45.5)	
Fasting glucose, mean mmol/L (SE)	5.70 (0.05)	5.27 (0.04)	5.90 (0.05)	7.11(0.24)	<0.001
HbA1c, mean% (SE)	6.1 (0.03)	6.0 (0.03)	6.1 (0.05)	6.5 (0.13)	<0.001

[a] Chi-square test for categorical data and Wald-test for continuous data.
[b] Fisher's exact test.

OGTT and the HbA_{1c} values. This regression technique provides separate regression coefficients for potential piecewise linear relations. To estimate the breakpoint between two segmented relations, the method uses information from the Davies' test for a non-zero difference in slope between variables. The HbA_{1c} values in this population ranged from 5% to 9%. Only five persons had HbA1c value above 7%. Although the corresponding glucose levels for the five patients were highly plausible, potential outliers may have influenced the estimation of cut-points in segmented regression. Therefore the segmented regression analyses were performed with and without these five subjects. No difference in estimated cut-points was found, suggesting that patients with an HbA1c value above 7% did not compromise the validity of the present segmented regression.

Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were used to evaluate the performance of HbA_{1c} when using the OGTT criteria as the gold standard. AUC was estimated for all study participants, including the subpopulation where HbA_{1c} values were measured within one month after the OGTT, and the subpopulation where HbA_{1c} values were measured within two months after the OGTT.

Results

Baseline characteristics of the study population are shown in Table 1. According to the OGTT criteria the prevalence of DM was 12% and the prevalence of intermediate hyperglycaemia was 33%.

The prevalence of reduced renal function was 20% in the normoglycaemic group, 34% in the intermediate hyperglycaemic group and 27% among the patients diagnosed with DM ($p = 0.04$). No statistically significant relation between reduced renal function and HbA_{1c} values was seen ($p = 0.46$). The majority of the study population was current or former smokers (80%). There were no significant differences in OGTT values and HbA_{1c} values with respect to vessels tested (carotid, aortic, iliac, infrainguinal) ($p = 0.16$). Separate analysis of glycaemic categories according to HbA_{1c} values revealed no statistically significant differences in age, sex, smoking status, renal function, anaemia, coronary heart disease or affected vascular bed.

Segmented regression analysis of FPG on HbA_{1c} values indicated a breakpoint at an HbA_{1c} value of 45 mmol/mol (6.3%) (95% CI 6.17, 6.51) in relation to FPG. Segmented regression analysis of OGTT 2-h level on HbA_{1c} values showed a breakpoint on HbA_{1c} value at 42 mmol/mol (6.0%) (95% CI 5.84, 6.18) in relation to OGTT 2-h value. These statistically derived breakpoints reveal a strong association between HbA_{1c} values and OGTT fasting plasma glucose values and OGTT 2 h-values at HbA_{1c}values 6.3% and 6.0% respectively,. This emphasises the integrity of the data.

ROC analysis showed an association between OGTT (gold standard) and HbA_{1c} values (test variable) as diagnostic parameters for DM with AUC 0.73 (95% CI 0.63, 0.84). AUC was independent of the difference in timing of blood sampling between OGTT results and HbA_{1c} values. There was no statistically significant association between OGTT results and HbA_{1c} values in the intermediate hyperglycaemia category (AUC 0.56) (Table 2).

Table 3 shows the number of patients categorized as having normoglycaemia, intermediate hyperglycaemia and DM according to HbA_{1c} values versus OGTT results, and the distribution of patients within the different glycaemic categories. According to OGTT results, 33 patients had DM, 90 patients had intermediate hyperglycaemia and 152 patients had normoglycaemia. Forty patients had DM according to HbA1c values. Fifteen patients were diagnosed with DM by both criteria, giving a sensitivity of 45.5%. The number of patients without DM by either criterion was 217 (Figure 2a). Ninety patients had intermediate hyperglycaemia according to OGTT results, and based on HbA_{1c} values these were classified similarly in 63% of the cases whereas 22% were grouped as having DM (Table 3, Figure 2b). According to HbA_{1c} criteria 193 patients had intermediate hyperglycaemia. The number of patients without intermediate hyperglycaemia by either criterion was 49 (Figure 2b).

HbA_{1c} values combined with FPG classified 46 patients as having DM, and diagnosed DM with a sensitivity of 64% and specificity of 90%.

Discussion

The purpose of this study was to validate HbA_{1c} as a method to diagnose DM in vascular surgery patients by comparing HbA_{1c} values with the OGTT results. In this study the prevalence of DM and intermediate hyperglycaemia was higher based diagnosis through measuring the HbA_{1c} values compared with OGTT results (Table 3). The two parameters defined the same individuals as having diabetes in only 45.5% of the cases (Table 3, Figure 2a). HbA_{1c} combined with FPG had a higher sensitivity (64%) compared to HbA_{1c} alone. There was a significant correlation between OGTT and reduced renal function ($p = 0.04$). No such correlation was seen for HbA_{1c}.

This was a prospective study with a well-defined study population of patients with advanced macrovascular disease. The selection of the study population was not based on FPG values. As a result, patients with normal FPG and elevated OGTT 2-h value were also diagnosed correctly. HbA_{1c} was measured in a standardised way to ensure the accuracy of the values measured. Medical conditions with abnormal red cell turnover may affect

Table 2 Area under curve and summary statistics of HbA_{1c} cut-off of 6.5 nmol/l for all patients, for patients with GFR ≥60 and for patients with GFR <60

Parameters	All patients	Where GFR ≥ 60	Where GFR < 60
No.	275	201	74
Area under curve (95% CI)	0.73 (0.63, 0.84)	0.71 (0.57, 0.84)	0.78 (0.63, 0.94)
Sensitivity (95% CI)	0.45 (0.28, 0.64)	0.46 (0.26, 0.67)	0.44 (0.14, 0.79)
Specificity (95% CI)	0.90 (0.85, 0.93)	0.92 (0.87, 0.96)	0.83 (0.72, 0.91)
Positive predictive value (95% CI)	0.38 (0.23, 0.54)	0.44 (0.24, 0.65)	0.27 (0.08, 0.55)
Negative predictive value (95% CI)	0.92 (0.88, 0.95)	0.93 (0.88, 0.96)	0.92 (0.81, 0.97)

HbA_{1c} measurement and give misleading results[1,22]. Haemoglobinopathies in Norway are mainly present in persons with African or Asian ethnic origin [23]. The study population was of European descent and there were no significant differences in the prevalence of anaemia, suggesting that anaemia and abnormal haemoglobin did not affect the validity of the results. The fact that 121 patients out of 467 declined to participate in the study might introduce a selection bias. However, the mean FPG values and the prevalence of DM based on FPG levels alone were the same in the 121 patients as in the study group.

Newly diagnosed DM

In contrast to most other studies an HbA_{1c} value ≥48 mmol/mol (6.5%) was associated with a higher prevalence of DM in this study when compared with OGTT results (14.6% vs. 12.0%) (Table 3). The sensitivity of HbA_{1c} compared with the OGTT in this study was 45.5%. Doerr et al performed coronary angiography in patients with coronary heart disease and found a lower prevalence of newly detected diabetes when using the HbA_{1c} criteria compared with the OGTT (4% vs. 14%) [20]. Results from major epidemiological studies on general populations also demonstrated a lower prevalence of diabetes by HbA_{1c} criteria compared with OGTT [14-16]. This corresponds with results from studies on populations at risk of developing DM [17,21]. A recent metaanalysis demonstrated that performing an OGTT during acute coronary syndrome did not impede the diagnostic accuracy of the test. The study did not compare HbA_{1c} with the OGTT [24].

Similar to the results presented in this paper, Mostafa et al found an increased prevalence of DM using HbA_{1c} criteria compared with the OGTT in a population based study on a multi-ethnic cohort[25]. Several explanations for the increased prevalence of DM by HbA_{1c} criteria in the population of vascular surgery patients may be presented. HbA_{1c} value measurements are influenced by high age, male sex, kidney failure and ethnicity [8,22,26], and pretest probability is influenced by the risk of DM development. All factors were present in this study, except that the study population consisted of white Europeans. On the contrary, Martins et al found that in older adults, females presented higher values of HbA_{1c} than men, and that HbA_{1c} is not affected by age[27]. Participants with IGT and/or DM were excluded.

Intermediate hyperglycaemia

An intermediate HbA_{1c} value range has been suggested to identify individuals in need for preventive interventions [8,9]. An interesting observation in this study is the high number of patients with intermediate hyperglycaemia as defined by HbA_{1c} in contrast to OGTT (70% vs. 33%) (Figure 2b). The prevalence of intermediate hyperglycaemia in the National Health and Nutrition

Table 3 The number of patients categorized as having normoglycaemia, intermediate hyperglycaemia and DM according to HbA_{1c} [a] versus OGTT[b] results, and the distribution of patients within the different glycaemic categories

OGTT[b]	Subjects	HbA_{1c} [a]		
		Normo-glycaemia (%)	Intermediate hyperglycaemia (%)	Diabetes mellitus (%)
Total	275	42 (15.3)	193 (70.2)	40 (14.6)
Normoglycaemia	152	27 (17.8)	120 (79.0)	5 (3.3)
Intermediate hyperglycaemia	90	13 (14.4)	57 (63.3)	20 (22.2)
Diabetes mellitus	33	2 (6.1)	16 (48.5)	15 (45.5)

[a] HbA_{1c} ≥ 6.5% (48 mmol/mol) = diabetes mellitus, HbA_{1c} range of 5.7-6.4% (39-46 mmol/mol) = intermediate hyperglycaemia, and HbA_{1c} < 5.7% (39 mmol/mol) = normoglycaemia.
[b] FPG + 2-h value. DM = FPG ≥ 7.0 mmol/l and/or two-h value ≥ 11.1 mmol/l. Intermediate hyperglycaemia = IGT defined as FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L, and IFG defined as fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h-value. Normoglycaemia = FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L.

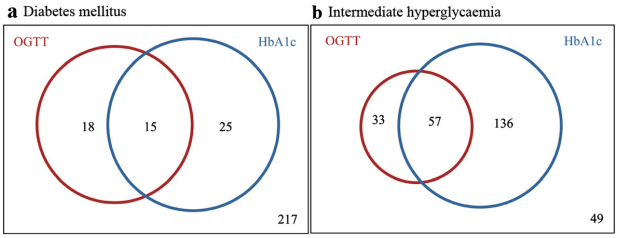

Figure 2 Venn diagrams. a The prevalence of newly diagnosed DM by OGTT results (FPG ≥ 7.0 mmol/l and/or 2-hour value ≥ 11.1 mmol/l) (red circle) and by HbA_{1c} value ≥ 6.5% (48 mmol/mol) (blue circle). The prevalence of diabetes mellitus was higher when using HbA_{1c} criteria, and HbA_{1c} classified 46% of the 33 patients with newly diagnosed diabetes mellitus in concordance with OGTT results. **b** The prevalence of intermediate hyperglycaemia by OGTT results (FPG 6.1 mmol/l – 7.0 mmol/l and/or 2-hour value 7.8 mmol/l – 11.1 mmol/l) (red circle) and by HbA_{1c} value 5.7% - 6.4% (39-46 mmol/mol) (blue circle). The prevalence of intermediate hyperglycaemia was higher when using HbA_{1c} criteria compared with OGTT results.

Examination Survey using HbA_{1c} criteria was one tenth that of OGTT results [15]. In contrast, among coronary heart disease patients the prevalence of intermediate hyperglycaemia using HbA_{1c} criteria was similar to that of OGTT results [20]. Major prospective studies indicate an increased risk of developing DM in patients within the intermediate hyperglycaemia range of HbA_{1c} [28-31].

Clinical interpretations

Existing literature is often challenging to interpret as previous studies were performed on various mixtures of study populations; populations with diabetes, populations without diabetes and mixed populations. This study was performed on patients with advanced macrovascular disease and a mixture of glucometabolic states. HbA_{1c} has been considered as a risk predictor for subsequent diabetes and microvascular disease [2-5,28], and has been proposed as a risk predictor for macrovascular disease in people with DM [6,29]. However, studies on patients with established coronary heart disease have shown that the OGTT and not HbA_{1c} is the best predictor for the macrovascular disease [20,32,33]. Both this and previously performed studies [14-17,20,21] have shown that the OGTT and HbA_{1c} define different categories of patients as having DM and intermediate hyperglycaemia. HbA_{1c} describes long-term glycaemic burden and represents a different metabolic expression to OGTT. The high prevalence of DM and intermediate hyperglycaemia when using HbA_{1c} in this study may reflect a high chronic glycaemic burden in patients with peripheral arterial disease.

The clinical importance of the high number of patients with DM and intermediate hyperglycaemia in this study with advanced macrovascular disease, whether defined by HbA_{1c} or OGTT, is unknown. Future studies are needed to identify which test, the OGTT or the HbA1c, is the better in predicting the clinical outcome in vascular surgery patients and in defining the patients in need for treatment or preventive intervention.

Limitations

The sample size of this study with participants from a specific high-risk population was relatively small. The discordance between OGTT results and HbA_{1c} values revealed the challenge when using a gold standard, i.e. that any other test will be inferior. Ideally, it would be preferable to use an external diagnostic and prognostic parameter, for example the prevalence of retinopathy, in order to compare HbA_{1c} with the OGTT. Due to logistic reasons, some OGTTs were not performed at standard conditions in the hospital for all patients. This reflects the clinical reality where the diagnosis of DM is mostly done by the primary health care provider. For the same reasons, the HbA_{1c} values were not measured at the same time as the OGTT for all patients. Separate analyses were performed on those patients who had their HbA_{1c} values measured between one month and two months after the time that their OGTT was performed. There were no significant differences in the results. It would have been preferable to use the mean of two fasting glucose levels, two two-hour glucose levels and two HbA_{1c} values for statistical analysis to secure reproducibility of the results. The International Expert Committee, American Diabetes Association and the WHO have suggested repeated HbA_{1c} measurements as a diagnostic criterion for type 2 DM [8-10]. However, large epidemiological studies have used the results from one single measurement of FPG, two-hour value and HbA_{1c}, thus making the results from this study comparable to other studies. Finally, it would have been preferable to have used body mass index as an adjustment variable, but this was not registered.

Conclusion

In vascular surgery patients an HbA_{1c} value of ≥48 mmol/mol (6.5%) had a 45.5% sensitivity, and a 90% specificity

when compared to the OGTT when diagnosing DM. The total prevalence of pathologic glucose metabolism was substantially higher based on HbA_{1c} values than based on OGTTs. The two parameters, the OGTT and the HbA_{1c}, categorized different individuals with DM and intermediate hyperglycaemia. The high prevalence of DM and intermediate hyperglycaemia when using HbA_{1c} values in this study may reflect a high chronic glycaemic burden in patients with peripheral arterial disease. Further studies on vascular surgery patients are needed to identify which method, the OGTT or the HbA_{1c}, is the better in predicting DM and future clinical development of vascular disease.

Abbreviations

AUC: Area under the curve; DM: Diabetes mellitus; FPG: Fasting plasma glucose; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; OGTT: Oral glucose tolerance test; PAD: Peripheral arterial disease; ROC: Receiver operating characteristic; WHO: World health organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ID Hjellestad: Contributed in the planning of the study and in the design of the study. Mainly responsible for literature search, contributed in analysis and interpretation of the results, principal author/drafted the manuscript. Mainly responsible for the VENN diagram figures. MC Astor: Contributed in the planning of the study, design of the study, interpretation of the results and preparation and critical revision of the manuscript. RM Nilsen: Mainly responsible for statistical analysis, contributed to the interpretation of the results and preparation and critical revision of the manuscript. E Søfteland: Contributed in the planning of the study, design of the study, interpretation of the results and preparation and critical revision of the manuscript. T Jonung: Contributed in the planning of the study, design of the study, interpretation of the results and preparation and critical revision of the manuscript. All authors read and approved the final manuscript.

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Cardiovascular Diabetology

CORRECTION

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Correction to: HbA_{1c} versus oral glucose tolerance test as a method to diagnose diabetes mellitus in vascular surgery patients

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Correction to: Cardiovasc Diabetol (2013) 12:79
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The authors found errors in Table 1 after publication of the original article [1].

The correct values for medical history of coronary artery disease (CAD) at baseline are 110 (40%) of all patients, 54(35.5%) of patients categorized as having normoglycaemia, 42(46.7%) of patients categorized as having intermediate hyperglycaemia, and 14 (42.4%) of patients categorized as having DM.

All presented numbers and calculations in Table 1 are checked. No other errors were found. The presented errors did not affect results, scientific content or conclusions.

The corrected Table 1 is presented in this erratum.

The authors apologize for having presented this error in the original article.

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Table 1 Baseline characteristics of the study population

Characteristics	All patients	OGTT			
		Normo-glycaemia	Intermediate hyperglycaemia	Diabetes mellitus	*P*-value[a]
Total	275	152	90	33	
Age, mean years [range]	69.5 [35–89]	68.0 [35–87]	71.5 [48–89]	71.1 [59–88]	0.01
Sex, *n* (%)					0.02
Female	74 (26.9)	51 (33.6)	15 (16.7)	8 (24.2)	
Male	201 (73.1)	101 (66.5)	75 (83.3)	25 (75.8)	
Smoking status, *n* (%)					0.06
Non-smoker	42 (15.3)	23 (15.1)	10 (11.1)	9 (27.3)	
Former/current smoker	221 (80.4)	123 (80.9)	77 (85.6)	21 (63.6)	
Missing	12 (4.36)	6 (3.95)	3 (3.33)	3 (9.09)	
Renal function, *n* (%)					0.04
Normal (eGFR > 60)	201 (73.1)	120 (79.0)	57 (63.3)	24 (72.7)	
Kidney failure (eGFR < 60)	71 (25.8)	31 (20.4)	31 (34.4)	9 (27.3)	
Missing	3 (1.09)	1 (0.66)	2 (2.22)		
Anemia female, *n* (%)					
No anemia	61 (82.4)	43 (84.3)	10 (66.7)	8 (100)	0.52[b]
Female Hb < 11.7 g/dL	6 (8.11)	4 (7.84)	2 (13.3)	0	
Missing	7 (9.46)	4 (7.84)	3 (13.3)	0	
Anemia male, *n* (%)					
No anemia	163 (81.1)	81 (81.2)	62 (82.7)	19 (76.0)	0.81[b]
Male Hb < 13.4 g/dL	29 (14.4)	16 (15.8)	9 (12.0)	4 (16.0)	
Missing	9 (4.48)	3 (2.97)	4 (5.33)	2 (8.00)	
Medical history of CAD, *n* (%)					0.34
No	165 (60.0)	98 (64.5)	48 (53.3)	19 (57.6)	
Yes	110 (40.0)	54 (35.5)	42 (46.7)	14 (42.4)	
Affected vascular bed, *n* (%)					0.16
Carotid	43 (15.6)	26 (17.1)	11 (12.2)	6 (18.2)	
Aortic	59 (21.5)	30 (19.7)	23 (25.6)	6 (18.2)	
IOD	50 (18.2)	35 (23.0)	9 (10.0)	6 (18.2)	
Infrainguinal	123 (44.7)	61 (40.1)	47 (52.2)	15 (45.5)	
Fasting glucose, mean mmol/L (SE)	5.70 (0.05)	5.27 (0.04)	5.90 (0.05)	7.11 (0.24)	< 0.001
HbA1c, mean % (SE)	6.1 (0.03)	6.0 (0.03)	6.1 (0.05)	6.5 (0.13)	< 0.001

[a] Chi square test for categorical data and Wald-test for continuous data

[b] Fisher's exact test

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Errata for
Biomarkers for Diabetes Mellitus in advanced Peripheral Arterial Disease

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Iren Drange Hjellestad

Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

24.04.19 Iren D. Hjellestad (date and sign. of candidate)

25.04.19 (date and sign. of faculty)

Errata

Paper I Missing units in Table 1: "eGFR >60" - should be "eGFR ≥ 60ml/min/1.73m2" and "eGFR < 60" – should be "eGFR > 60ml/min/1.73m2".

Paper I Misplaced reference to Table 2 in the Results, page 5, second column, para 2: "(Table 2)" should be placed at the end of the first sentence, second column, para 2, "….. AUC 0.73 (95% CI 0.63,0.84) (Table 2)".

Paper I Incorrect HbA_{1c} unit in Table 2: "6.5nmol/L" – correct unit should be " 6.5%".

Paper I Missing letters and units in Table 2: "GFR ≥ 60" – should be "eGFR ≥ 60ml/min/1.73m2" and "GFR< 60" – should be "eGFR < 60ml/min/1.73m2".

II

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Abdominal aortic aneurysms – glycaemic status and mortality

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ABSTRACT

Aims: The prevalence of diabetes mellitus (DM) and mortality with respect to glycaemic status in patients with abdominal aortic aneurysms (AAA) was evaluated. Glycaemic status was assessed by an oral glucose tolerance test (OGTT) and by HbA_{1c}.
Methods: Sixty-six patients with AAA admitted to the vascular surgery unit for elective surgery between October 2006 and September 2007 were included. Seven patients had previously known DM. OGTT and HbA_{1c} results were available from 58 patients. The patients were categorized as having DM, prediabetes and normoglycaemia according to the WHO's and American Diabetes Association's criteria.
Results: The prevalence of newly diagnosed DM according to the OGTT and HbA_{1c} results were 12% and 14% respectively. Mean follow-up time was 68 months and all-cause mortality 43%. HbA_{1c} was an independent predictor for mortality in the DM category. Hazard ratio of all-cause mortality in the DM category defined by the HbA_{1c} values was 6.35, 95% [CI 1.49–27.1]; $p = 0.01$.
Conclusions: DM defined by $HbA_{1c} \geq 6.5\%$ is an important determinant of mortality following surgical treatment for AAA. Half the patients with AAA and DM were unaware of their DM diagnosis. All patients with AAA should be tested for DM using HbA_{1c}. The results should be confirmed in a larger prospective study.

1. Introduction

The diagnosis of diabetes mellitus (DM) is defined as either fasting plasma glucose levels (FPG) ≥ 7.0 mmol/L, and/or two-hour oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L or an HbA_{1c} value of ≥ 48 mmol/mol (6.5%) (American Diabetes Association, 2010; World Health Organization, 2006).

Previous studies have revealed a higher DM prevalence in patients with peripheral arterial disease (PAD) (Astor, Softeland, Daryapeyma, & Jonung, 2010; Selvin & Erlinger, 2004) compared to general populations (Cowie et al., 2006; Midthjell & CMYLCPSC, 2010) and populations at risk of developing DM (Peter et al., 2011).

When comparing the diagnostic modalities for the diagnosis of DM (HbA_{1c} and the OGTT including FPG and/or 2 h-post glucose value), it has been shown that they to a significant degree define different individuals as having DM (Cowie et al., 2010; Doerr et al., 2011; Hjellestad, Astor, Nilsen, Softeland, & Jonung, 2013; Lauritzen, Sandbaek, Skriver, & Borch-Johnsen, 2011; Midthjell & CMYLCPSC, 2010; Peter et al., 2011; Rathmann et al., 2012). In a cohort of patients with peripheral arterial disease located in western Norway, the total prevalence of pathologic glucose metabolism was substantially higher when based on HbA_{1c} values than when based on the OGTT. The two parameters largely defined different patients with DM (Hjellestad et al., 2013). No studies could be found comparing the prevalence of DM by the two criteria in patients with abdominal aortic aneurysm (AAA).

Up to one third of patients with type 2 DM are unaware of their DM diagnosis (World Health Organization, 2006), and are therefore untreated and at high risk of developing vascular complications. Patients with AAA and DM defined by OGTT have a higher risk of complications and shorter long-term survival compared with AAA patients without known DM - although studies have reported inconsistent results (Theivacumar, Stephenson, Mistry, & Valenti, 2014). Further, the impact of DM and prediabetes on mortality in patients with AAA, based on the OGTT and the HbA_{1c} values, is not known.

It is of vital importance to inform about the high level of dysglycaemia in AAA patients to improve diagnostics and treatment of dysglycaemia and improve clinical outcome.

The aim of this study was to evaluate the prevalence of DM and prediabetes in AAA patients, and the mortality with respect to glycaemic status assessed by OGTT and HbA_{1c}.

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2. Methods

2.1. Patient Selection

This study was a prospective cohort study including patients with abdominal aortic aneurysms admitted for surgical treatment for their AAA. The initial study population consisted of 345 patients admitted consecutively to the vascular surgery unit for elective surgery between October 2006 and September 2007. The vascular pathologies were carotid stenosis, AAA, iliac occlusive disease and infrainguinal occlusive disease. Sixty-six of these patients had AAA, and were included in the present study. Seven patients had previously known DM. HbA_{1c} value was missing in one patient. An OGTT was performed on the remaining 58 patients and the results were included for further analysis. Fasting glucose and HbA_{1c} values were analyzed in all patients. The research protocol was approved by the Regional Committee for Medical Research Ethics (REK vest 14109).

2.2. Diagnostic Tests

An OGTT was performed on 58 patients. Fasting glucose and HbA_{1c} values were measured in all participants. The OGTT was performed by orally administering a standard dose of 75 g anhydrous glucose dissolved in water after a minimum of eight hours overnight fasting. Plasma glucose levels were measured in a fasting state prior to administering the anhydrous glucose and again two hours after administration. Venous whole blood, drawn in containers with glycolytic inhibitors (citrate and fluoride) and centrifuged within one hour from venous sampling to separate plasma, was used for the OGTT glucose measurements.

The OGTT plasma glucose levels were analysed using Modular P (Roche Diagnostics). The OGTT results were categorized into three groups according to the WHO 1999 criteria: 1/DM defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/l and/or two-hour value (2-h-value) ≥ 11.1 mmol/l, 2/prediabetes, which consists of IGT defined as FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L, and IFG defined as fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h-value and 3/normal glucose metabolism defined as FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L.

HbA_{1c} values were measured in all participants through a single blood sample using Variant II HPLC system (Biorad). The HbA_{1c} results were categorized as: DM, prediabetes and normoglycaemia. The diagnostic limit of HbA_{1c} is ≥48 mmol/mol (6.5%) according to the WHO statement of 2011. The American Diabetes Association definition of prediabetes at 39–46 mmol/mol (5.7–6.4%) was used since the WHO has not yet made a statement on the HbA_{1c} diagnostic range of prediabetes.

External quality assessment of all equipment used for analysis was performed by NOKLUS. NOKLUS is a national institution certified by The National Institute of Technology (NS-EN ISO 9001:2000), and run by a committee consisting of representatives from The Norwegian Health Ministry, The Norwegian Medical Association and The Norwegian Association of Local and Regional Authorities. NOKLUS is quality checked by The European Reference Laboratory for Glycohemoglobin in the Netherlands.

2.3. Other Variables

Information about age (continuous), sex (men/women), smoking habits (yes/no), aortic diameter (continuous), body mass index (BMI) (kg/m^2), state of anaemia (yes/no), reduced renal function (yes/no), coronary artery disease (yes/no), cerebrovascular disease (yes/no), established peripheral occlusive disease (yes/no) and medical treatment (the use of statins, platelet inhibitors and antihypertensive medication) (yes/no) at time of inclusion was obtained from the patients' medical records. The presence of reduced renal function was defined based on estimated glomerular filtration rates (eGFR), calculated by The Modification of Diet in Renal Disease Equation.

Surgical treatment for AAA was either open surgery or an endovascular procedure. Patients with high age and comorbidities were more likely to be treated with an endovascular procedure while younger patients and patients without comorbidities were more likely to be treated with open surgery.

2.4. End Points and Follow-Up

The study was closed on the 11th of August 2014. Mean follow-up time of the study population was 71 months [range: 0–100]. Follow-up time for each participant was defined as the number of months from the date of surgical intervention to the date of death, or to the date of study closure. Mortality rates were verified by the Norwegian civil registry. The primary end point was death of all cause.

2.5. Statistical Analysis

Data were analysed using R version 3.2.1 (R Core Team, 2015) software for Windows. All p values were 2-sided, and values <0.05 were considered statistically significant. The data were presented as mean ± standard error for continuous data, and as number and percentage for categorical data. Associations between categorical variables were analysed using χ^2 test. When the expected number of observations in one or more categories was ≤ 5, the Fisher's exact test was used. The Kaplan–Meier survival function was used to describe the percentage of survivors since study inclusion. To test for difference in survival functions across glycaemic categories based on both the OGTT and the HbA_{1c} value, the log-rank test was used.

Associations of OGTT and HbA_{1c} glycaemic categories with all-cause mortality were further estimated as hazard ratios with 95% confidence intervals (CIs) using Cox regression models. The time from study inclusion until death was used as the measure of event free time. All patients were monitored until censoring with 11th of August 2014 as the final day of follow-up. The hazard ratios were estimated by crude models as well as after controlling for age, platelet inhibitor and statin. The three covariates were selected among 11 covariates, measured at study inclusion, by using a stepwise method stated in literature (Collet, 2015). The covariates initially explored were age, the use of platelet inhibitor, BMI, sex, hypertension, reduced renal function, coronary artery disease, smoking status, the use of statin, the use of antihypertensive medication and established peripheral occlusive disease. Based on a test and inspection of scaled Schoenfeld residuals, it was verified that the proportional-hazards assumption was fulfilled for all variables in the final models.

3. Results

Baseline characteristics of all participants are presented in Table 1. The study population was an elderly population with a high prevalence of comorbidities. The majority of the participants were former or current smokers. Patients who died during follow-up had significantly higher age and more often a history of cerebrovascular disease at baseline. They were more likely to be treated with an endovascular procedure than open surgery in comparison with patients who were alive at end of the study.

3.1. Glycaemic Status

The prevalence of newly diagnosed DM, prediabetes and normoglycaemia, in patients with AAA is summarized in Table 2. The HbA_{1c} criteria and the OGTT results largely classified different patients as having newly diagnosed DM. The total prevalence of DM in this

Table 1
Baseline characteristics.

Characteristics	All patients	Patients without former known DM	Patients dead during follow up	Patients alive at end study	*p*-value[a]
Total	66	58	25	33	
Age, mean years	71.4	71.3	74.0	69.2	0.004
[range]	[59–86]	[59–85]	[61–83]	[61–83]	
Sex, *n* (%)					
Female	8 (13.1)	4 (6.9)	1 (4.0)	3 (9.1)	n.s.[b]
Male	53 (86.9)	54 (93.1)	24 (96.0)	30 (90.9)	n.s.
BMI kg/m^2 (SE)	26.1 (0.60)	26.1 (0.60)	25.7 (1.03)	26.5 (0.71)	n.s.
[range]	[17.6–36.1]	[17.7–36.1]	[17.7–36.1]	[19.7–33.5]	
Missing	10				
Aortic diameter, mean cm [range]	6.13	6.02	6.17	5.99	n.s.
	[4.50–9.3]	[4.50–8.90]	[5.50–8.10]	[4.40 –8.90]	
Type of operation					
Open, *n* (%)	40 (60.6)	35 (60.3)	10 (40.0)	25 (75.8)	0.006
Endovascular, *n* (%)	25 (37.9)	22 (37.9)	15 (60.0)	7 (21.2)	0.003
Conservatively treated, *n* (%)	1 (1.5)	1 (1.7)	0	1 (3.0)	
Smoking status, *n* (%)					
Non-smoker	13 (19.7)	10 (17.2)	4 (16.0)	6 (18.2)	n.s.[b]
Former/Current smoker	51 (77.3)	47 (81.1)	21 (84.0)	26 (78.8)	n.s.
Missing	2 (3.0)	1 (1.7)	0	1 (3.0)	
Renal function, *n* (%)					
Normal (eGFR > 60)	49 (74.2)	43 (74.1)	17 (68.0)	26 (78.8)	n.s
Reduced (eGFR < 60)	17 (25.8)	15 (25.9)	8 (32.0)	7 (21.2)	n.s
Missing	0	0	0	0	
Medical history of hypertension, *n* (%)					
No	38 (57.6)	31 (53.4)	12 (48.0)	19 (57.6)	n.s.
Yes	28 (42.4)	27 (46.6)	13 (52.0)	14 (42.4)	n.s.
Missing	0	0			
Medical history of CVD, *n* (%)					
No	48 (72.7)	41 (70.7)	14 (56.0)	27 (81.8)	0.03
Yes	18 (27.3)	17 (29.3)	11 (44.0)	6 (18.2)	0.03
Missing		0			
Medical history of CAD, *n* (%)					
No	31(47.0)	29 (50.0)	11 (44.0)	18 (54.5)	n.s.
Yes	35(53.0)	29 (50.0)	14 (56.0)	15 (45.5)	n.s.
Missing	0	0	0		
Fasting glucose, mean mmol/L	6.1 (0.22)	5.9 (0.14)	5.93(0.16)	5.92 (0.21)	n.s.
(SE)	[4.5–18.1]	[4.5–10.5]	[4.5–8.4]	[4.6–10.5]	
HbA1c, mean %	6.2 (0.09)	6.1 (0.06)	6.2 (0.86)	6.0 (0.08)	n.s.
(SE) [range]	[5.1–9.3]	[5.1–7.9]	[5.5–7.0]	[5.1–7.9]	

CAD = coronary artery disease, CVD = cerebrovascular disease.
[a] Chi-square test. *p*-value refers to the association between patients dead during follow up and patients alive at the closure of the study.
[b] Fisher's exact test.

population was 23% based on the OGTT results and 25% based on the HbA_{1c} levels.

3.2. Mortality

Mean follow-up time was 71 months [range 0–100]. All-cause mortality among the patients without previously diagnosed DM was 43%. The HbA_{1c}, and not the OGTT, was a significant independent predictor for mortality in the DM category (Fig. 1). Final adjusted hazard ratio of all-cause mortality in the DM category defined by the HbA_{1c} values was 6.35, 95% [CI 1.49–27.1]; $p = 0.01$ (Table 3). According to the OGTT results, patients with prediabetes and not with DM had a significantly higher mortality rate during the course of the study in crude analysis, HR 2.83, [CI 1.16–6.89]; $p = 0.02$. This association did not achieve statistical significance after multivariate analysis HR 2.28, [CI 0.84– 6.15]; $p = 0.10$ (Table 3).

Table 2
The number of patients with AAA categorized as having normoglycaemia, prediabetes and diabetes mellitus according to HbA_{1c}[a] versus OGTT[b] results.

HbA_{1c}[a]					
		Normo-glycaemia n (%)	Prediabetes n (%)	Diabetes mellitus n (%)	Total n (%)
OGTT[b]	Normo-glycaemia	4 (15.4)	21 (80.8)	1 (3.8)	26 (44.8)
	Prediabetes	3 (12.0)	18 (72.0)	4 (16.0)	25 (43.1)
	Diabetes mellitus	1 (14.3)	3 (42.9)	3 (42.9)	7 (12.1)
	Subjects	8 (13.8)	42 (72.4)	8 (13.8)	58

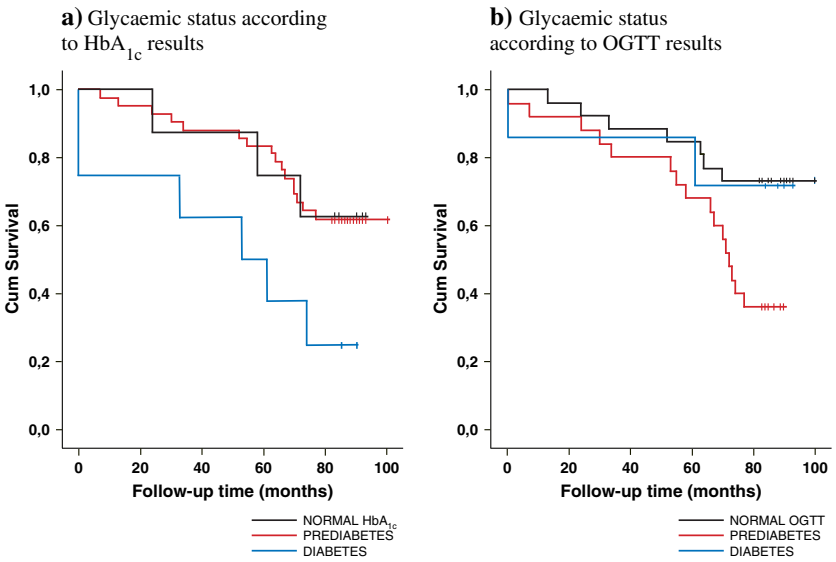
[a] $HbA_{1c} \geq 6.5\%$ (48 mmol/mol) = diabetes mellitus, HbA_{1c} range of 5.7–6.4% (39–46 mmol/mol) = prediabetes, and $HbA_{1c} < 5.7\%$ (39 mmol/mol) = normoglycaemia.
[b] FPG + 2-h value.

3.3. Medical Treatment

At time of inclusion, 85% of the study population received statins, 76% antiplatelet therapy, 10% warfarin and 86% received antihypertensive treatment. Patients who were treated with antiplatelet therapy at baseline had a significantly higher survival rate than those not treated. Only one patient with newly diagnosed DM using OGTT results received medical treatment for DM at the closure of the study. No patients with newly diagnosed DM by HbA_{1c} results received medical treatment for DM at the closure of the study.

4. Discussion

The aim of this study was to evaluate the prevalence of DM, prediabetes and mortality with respect to glycaemic status in patients with AAA, by using the OGTT and the HbA_{1c} values.



Hazard ratio for death by Cox regression model in the DM category by HbA_{1c} results:

Univariate: HR death 3.19, 95% [CI 0.80 -12.8]; p 0.10

Multivariate: HR death 6.35, 95% [CI 1.49 - 27.1]; p 0.01

Hazard ratio for death by Cox regression model in the prediabetes category by OGTT results:

Univariate: HR death 2.83, 95% [CI 1.16 -6.89]; p 0.02

Multivariate: HR death 2.28, 95% [CI 0.84 - 6.15]; p 0.10

Fig. 1. Kaplan–Meyer estimation of diabetes related survival curves in patients grouped according to HbA_{1c} results[c] (a) and OGTT results[d] (b). [c] HbA_{1c} ≥ 6.5% (48 mmol/mol) = diabetes mellitus, HbA_{1c} range of 5.7–6.4% (39–46 mmol/mol) = prediabetes, and HbA_{1c} < 5.7% (39 mmol/mol) = normoglycaemia. [d] FPG + 2-h value. DM = FPG ≥ 7.0 mmol/l and/or two-h value ≥ 11.1 mmol/l. Prediabetes = IGT defined as FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L, and IFG defined as fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h-value. Normoglycaemia = FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L.

The results from this study provide new information about glycaemic status and mortality in patients with AAA, and may have implications for the medical treatment of diabetes and clinical outcome in patients with AAA.

In this study, the prevalence of newly diagnosed DM according to the OGTT and the HbA_{1c} results were 12% and 14% respectively. The total prevalence of DM in the study population was 23% based on the OGTT results and 25% based on the HbA_{1c} values. Mean follow-up time was 71 months and all-cause mortality 43%. The HbA_{1c}, and not the OGTT, was a significant independent predictor for mortality in patients with DM (Table 3). This study is, probably, the first to describe long-term mortality in patients with AAA with respect to glycaemic status using both OGTT and HbA_{1c} values.

4.1. Methodological Considerations

This study was a prospective cohort study of a well-defined population of patients with AAA admitted for surgical intervention.

Table 3
Hazard ratio for death according to different glycaemic categories.

Glycaemic categories	No. of subjects	No. of deaths	Crude analysis[a]			Adjusted analysis[b]		
	N	*n (%)*	*HR*	*95% CI*	*p value*	*HR*	*95% CI*	*p value*
HbA_{1c} results[c]								
Normoglycaemia[d]	8	3 (37.5)	1.00			1.00		
Prediabetes	42	16 (38.1)	1.00	0.29, 3.44	0.99	0.73	0.21, 2.58	0.62
Diabetes mellitus	8	6(75.0)	3.19	0.80, 12.8	0.10	6.35	1.49, 27.1	0.01
OGTT results[e]								
Normoglycaemia[d]	26	7 (26.9)	1.00			1.00		
Prediabetes	25	16 (64.0)	2.83	1.16, 6.89	0.02	2.28	0.84, 6.15	0.10
Diabetes mellitus	7	2 (28.6)	1.12	0.23, 5.40	0.87	1.98	0.39, 10.1	0.41

[a] By cox regression model.
[b] Adjusted for age, platelet inhibitor and statin.
[c] HbA_{1c} ≥ 6.5% (48 mmol/mol) = diabetes mellitus, HbA_{1c} range of 5.7–6.4% (39–46 mmol/mol) = prediabetes, and HbA_{1c} < 5.7% (39 mmol/mol) = normoglycaemia.
[d] Reference category.
[e] Fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or two-hour value (2-h-value) ≥ 11.1 mmol/L = diabetes mellitus; IGT (FPG < 7.0 mmol/L and a 2-h-value between 7.8 mmol/L and 11.1 mmol/L), and IFG (fasting glucose value between 6.1 mmol/L and 7.0 mmol/L with a normal 2-h-value) = prediabetes; and FPG < 6.1 mmol/L and a 2-h-value < 7.8 mmol/L = normal glucose metabolism.

The follow-up period was up to six years. No patients were lost to follow-up. The high percentage of male gender, and comorbidities such as coronary artery disease, hypertension, cerebral arterial disease, in the study material was comparable to the findings in previous studies on subjects with AAA (Cornuz, Sidoti, Tevaearai, & Egger, 2004; Kanagasabay, Gajraj, Pointon, & Scott, 1996; Lederle et al., 1997; Long et al., 2010). This indicates that the study material was representative for subjects with AAA, although the sample size was small. All patients were tested for DM using both the OGTT and the HbA_{1c} values, hence no patients were preselected with respect to glycaemic status. The OGTT and the HbA_{1c} were measured in a standardized way for all patients.

In this study, patients categorized as having DM by OGTT values, were informed about their DM diagnosis. At the time of inclusion in the study, HbA_{1c} was not yet implemented as a diagnostic parameter for the diagnosis of DM, hence patients with $HbA_{1c} \geq 6.5\%$ and OGTT results below the diagnostic limit of DM, were not informed about the result. As a consequence, patients with DM by the OGTT criteria may have received treatment against DM in contrast to patients with $HbA_{1c} \geq 6.5\%$ and an OGTT result below the diagnostic limit of DM. This may have influenced the association of mortality with respect to the OGTT categories. However, a review of the patients' medical records showed that only one patient with newly diagnosed DM by the OGTT results received medical treatment for DM at the closure of the study. Also the mean HbA_{1c} value in patients with DM categorized by the OGTT results was below 7.0% which is the recommended target value of HbA_{1c} in the treatment of diabetes. Further the HbA_{1c} criteria classified three of the seven patients with newly diagnosed DM in concordance with the OGTT results. The remaining five patients diagnosed with DM by HbA_{1c}, but not by OGTT results could introduce a confounding factor as they did not receive information about their glycaemic status. The results in this study were limited to patients with AAA, and are not necessarily applicable on general populations or populations with other vascular pathologies. The statistics in this study is affected by a small sample size. Therefore it is recommended that a larger scaled prospective study on patients with AAA should be performed to confirm these results.

4.2. Clinical Considerations

The results from this study indicate that patients with AAA and DM diagnosed using HbA_{1c} values have significantly higher mortality rates compared with AAA patients with non-diabetic HbA_{1c} values. This association was not found in patients with DM by OGTT results when compared to normal OGTT values. According to the OGTT results patients with *prediabetes* showed a significantly higher mortality rate compared to normoglycaemic patients in a univariate analysis, but does not follow multivariate adjustment. Due to small sample sizes in the subgroups of glycaemic categories, the possibility of a type two error cannot be excluded. However, the mortality rates in relation to the OGTT results in this study are comparable to the findings of van Kuijk et al. in patients with peripheral arterial disease (van Kuijk et al., 2009). De Rango et al found that operative and long-term survival is lower in patients with AAA and DM compared to patients with AAA alone, suggesting an increased cardiovascular burden in patients with AAA and DM (De, Farchioni, Fiorucci, & Lenti, 2014).

Former studies on AAA have, surprisingly, suggested a protective role of DM against the development of AAA based on the observation that the prevalence of AAA is lower in diabetic populations compared with general populations (LaMorte, Scott, & Menzoian, 1995; Lederle et al., 1997; Mattes et al., 1997; Shah et al., 2014). The studies have not provided evidence for the pathomechanisms behind these observations, but glycation of proteins in the media layer of the abdominal aortic wall and the medical treatment of patients with DM have been proposed as possible explanations to the lower prevalence of AAA in diabetic populations (Shantikumar, Ajjan, Porter, & Scott, 2010). The referred to studies had a preselected study population of patients with diagnosed DM defined by glucose criteria. Patients with DM who are unaware of their DM diagnose and patients with DM defined by HbA_{1c} values are thereby excluded from these studies and may represent a selection bias. This may lead to a falsely low prevalence of AAA in patients with DM in these studies. The results from this study on Norwegian patients with AAA revealed a total prevalence of newly diagnosed and previously known DM according to the OGTT and the HbA_{1c} were 23% and 25% respectively. The prevalence of DM in the general Norwegian population is reported to be 8% at a comparable category of age (Stene et al., 2004).

4.3. The Role of HbA_{1c}

A large European study on patients with coronary artery disease (CAD) compared values of FPG, 2-h post glucose load and HbA_{1c} values when diagnosing DM, and found that in patients with established CAD, the OGTT defined the largest number of patients with DM. The authors recommended that the OGTT, and not the HbA_{1c}, should be the preferred diagnostic test for the diagnosis of DM in patients with CAD (Gyberg et al., 2015). The study had a cross-sectional design and thus could not provide information about the impact of the two diagnostic criteria for diabetes on long-term mortality. The results of this study indicated that HbA_{1c}, and not the OGTT, was associated with long-term all-cause mortality in patients with AAA. Patients with AAA have a high degree of vascular comorbidities, especially CAD and peripheral occlusive disease (Cornuz et al., 2004; Kanagasabay et al., 1996; Lederle et al., 1997; Long et al., 2010). An association between glycaemic control and systemic inflammation in patients with established DM has been shown both in the diabetic and non-diabetic ranges of HbA_{1c} (Gustavsson & Agardh, 2004; King, Mainous, Buchanan, & Pearson, 2003; Marques-Vidal et al., 2013). Gustavsson et al. found even an association of inflammation with HbA_{1c} values in patients with CAD within the normal range of HbA_{1c} values.

Large epidemiological studies have proven the association of both OGTTs results and HbA_{1c} values with the development of micro-and macrovascular disease and mortality (Alssema et al., 2011; Andersson et al., 2012; McCance et al., 1994; Miyazaki et al., 2004; Selvin et al., 2004; Skriver, Borch-Johnsen, Lauritzen, & Sandbaek, 2010). Recent studies on diabetes have shown that the OGTT and the HbA_{1c} criteria largely define different patients as having DM (Cowie et al., 2010; Doerr et al., 2011; Hjellestad et al., 2013; Lauritzen et al., 2011; Midthjell & CMYLCPSC, 2010; Peter et al., 2011; Rathmann et al., 2012). This is in concordance with findings in this study on patients with AAA. The OGTT and the HbA_{1c} represent different metabolic expressions. The OGTT is a stress-test of the function of pancreatic islet cells whereas HbA_{1c} represents long term exposure to plasma glucose.

HbA_{1c} may to a greater extent than OGTT express the degree of macro- and microvascular inflammation. This could be a possible explanation of the association between HbA_{1c} and all-cause mortality in this study. This study was, however, not designed to give an answer to this assumption.

4.4. Prediabetes

According to the American College of Cardiology/American Heart Association guidelines for the treatment of peripheral vascular disease, DM is recognized as an atherosclerotic risk factor (Anonymous, 2003). Notable is the observation of the high prevalence of prediabetes according to HbA_{1c} values in this study (Table 2). Results show a trend toward higher mortality in patients with prediabetes compared with patients with normal HbA_{1c} values. The results were, however, not statistically significant. The results may indicate that patients with AAA and prediabetes according to HbA_{1c} values should be included in a postoperative outpatient program to prevent and monitor the development of DM.

5. Conclusion

DM defined by $HbA1_c \geq 6.5\%$ is an important determinant of mortality following surgical treatment for AAA. Patients with AAA have high prevalence of DM and high all-cause mortality. Half the patients with AAA and DM are unaware of their DM diagnosis. Patients with DM as defined by HbA_{1c} values have a significantly higher mortality compared to normoglycaemic AAA patients. The results of this study indicate that all patients with AAA should be tested for DM by using the HbA_{1c}, and the results may have implications on medical treatment of patients with AAA. The results should, however, be confirmed in a larger prospective study and, if verified, followed by a prospective intervention study targeting glucose control.

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ORIGINAL ARTICLE

HbA1c predicts long-term postoperative mortality in patients with unknown glycemic status at admission for vascular surgery: An exploratory study

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Highlights

- Mortality during a 9-year follow-up of patients with peripheral arterial disease was 40%.
- HbA1c is a useful marker in preoperative risk assessment of patients with unknown glycemic status at admission for vascular surgery.

Abstract

Background: Peripheral arterial disease (PAD) and diabetes mellitus (DM) represent major public health challenges and are tightly associated. To facilitate early diagnosis, HbA1c has been implemented as the preferred diagnostic tool for the diagnosis of type 2 DM. In this study, we compared and evaluated HbA1c, fasting plasma glucose (FPG), and 2-hour post-load glucose values to determine which test best predicted mortality in patients with PAD.

Methods: In all, 273 PAD patients with unknown glycemic status admitted to Haukeland University Hospital for elective surgery between October 2006 and September 2007 were included in the study. All 273 patients underwent a standard oral glucose tolerance test (OGTT) in addition to determination of HbA1c; patients were then grouped into those with DM, intermediate hyperglycemia, and normoglycemia according to World Health Organization and International Expert Committee criteria.

Results: All-cause mortality was 40% over a 9-year follow-up period. After adjusting for age, sex, and relevant medication, HbA1c was a predictor for mortality (hazard ratio [HR] 1.54; 95% confidence interval [CI] 1.03-2.32]; $P = 0.04$). The association did not achieve statistical significance in a fully adjusted Cox regression model, although the effect estimation of HbA1c on all-cause mortality remained largely unchanged (HR 1.39; 95% CI 0.92-2.09; $P = 0.13$). The OGTT was not a predictor of long-term mortality.

Conclusions: The results indicate that HbA1c is a useful marker in the preoperative screening of patients of unknown glycemic status at the time of admission for vascular surgery, and may identify people at high risk of long-term mortality following surgical treatment for PAD.

KEYWORDS

diabetes, HbA1c, mortality, peripheral arterial disease

This study is registered with the Norwegian Regional Committee for Medical and Health Research Ethics (REK VEST), ID: 14109, 01/2006.

1 | INTRODUCTION

Peripheral arterial disease (PAD) and diabetes mellitus (DM) are tightly associated.[1] Based on fasting plasma glucose (FPG) levels, an oral glucose tolerance test (OGTT), or a self-reported DM diagnosis, patients with PAD are reported to have a higher prevalence of DM compared with general populations and populations at risk of developing DM.[2–6] In Norwegian vascular surgery patients, a 55% prevalence of hyperglycemia and a DM frequency of 29% were found as defined by FPG and an OGTT.[7]

The diagnostic criteria for DM have changed over time along with the development and improvement in biochemical tests. The diagnosis of diabetes is currently based on either an FPG ≥7.0 mmol/L, a 2-hour post-load glucose (2hPG) ≥11.1 mmol/L, or HbA1c ≥48 mmol/mol (6.5%).[8–10]

Studies have shown that FPG, 2hPG, and HbA1c levels primarily define different groups of patients as having DM.[4,11–13] In addition, patients with PAD and DM, defined by FPG or an OGTT, have higher mortality than patients with PAD alone.[1,14,15]

It is of major importance to investigate the consequences of unknown DM in PAD patients when using the current three diagnostic criteria for the DM diagnosis. The aim of the present study was to compare HbA1c, FPG and 2hPG values and to evaluate which test best predicts long-term mortality in patients with PAD.

2 | METHODS

This study is a prospective cohort study of patients with unknown glycemic status at the time of admission for vascular surgery.

2.1 | Study population

Initially, 465 patients admitted to Haukeland University Hospital for elective surgery between October 2006 and September 2007 were invited to participate in the study (Figure 1). Twenty-one patients died before deciding whether to participate, 66 declined to undergo an OGTT, and 33 did not have an OGTT performed due to logistic reasons. Furthermore, a DM diagnosis had been previously established in 67 patients. Thus, complete data on HbA1c, FPG, and 2hPG values from 273 vascular surgery patients were included for statistical analyses in the present study with unknown glycemic status (Figure 1). The vascular pathologies were carotid stenosis, abdominal aortic aneurysm (AAA), iliac occlusive disease (IOD) including lesions in the common femoral artery, and infrainguinal occlusive disease. Informed written consent was obtained from all participants. The research protocol was approved by the Norwegian Regional Committee for Medical and Health Research Ethics (REK vest 14109).

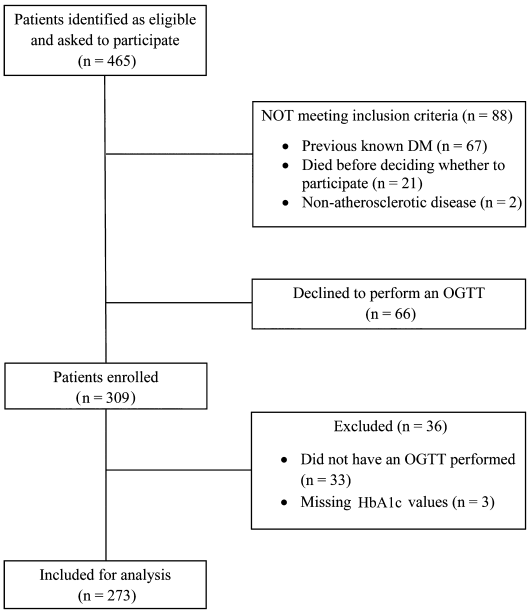

FIGURE 1 Flow-chart showing the selection of study participants admitted to Haukeland University Hospital, Norway, for elective vascular surgery between October 2006 and September 2007. DM, diabetes mellitus; OGTT, oral glucose tolerance test

2.2 | Inclusion and exclusion criteria

All patients admitted for elective vascular surgery due to atherosclerotic disease and competent to give consent to participate in the study were considered eligible for inclusion. Patients admitted for emergency procedures, patients with non-atherosclerotic conditions, patients with dementia or mental disability, and patients with known DM were excluded from the study.

2.3 | Diagnostic tests

Throughout this paper, the term "OGTT" refers to the measurements of both FPG and 2hPG, unless stated otherwise. The OGTT was performed according to World Health Organization (WHO) guidelines.[8] Plasma glucose concentrations were measured in a fasting state and again 2 hours after the administration of a 75-g glucose load. Diet recommendations were not given prior to the OGTT and HbA1c measurements. Plasma glucose concentrations were primarily assayed using Modular P analytical system (Roche Diagnostics). The HbA1c values were measured in samples of venous whole blood and analyzed using a Variant II HPLC system (Bio-Rad). The diagnostic tests have been described in detail elsewhere.[16]

The OGTT results were categorized according to 2006 WHO criteria,[8] whereas HbA1c results were categorized

according to the 2011 WHO statement[10] and the International Expert Committee statement of 2009.[17] Diabetes was defined as FPG ≥7.0 mmol/L, 2hPG ≥11.1 mmol/L, or HbA1c ≥48 mmol/mol (6.5%). Patients at high risk of developing DM were defined as those with HbA1c in the range 42 to 46 mmol/mol (6.0%-6.4%), FPG between 6.1 and 7.0 mmol/L, and 2hPG between 7.8 and 11.1 mmol/L.

2.4 | Other variables

Baseline data on age, sex, smoking habits, vascular bed affected, cholesterol levels, medication, and comorbidities were obtained from patients' medical records. The Modification of Diet in Renal Disease equation was used to calculate the estimated glomerular filtration rate (eGFR).[18] Reduced renal function was defined as eGFR <60 mL/min per 1.73 m^2.

2.5 | Endpoints and follow-up

Study participants were followed from the date of surgical intervention either to the date of death or to the date of study closure on 30 August 2016. Data on all-cause mortality were obtained from the Norwegian civil registry. Death of any cause was used as the primary endpoint. An autopsy was performed only on one-third of patients who died during the follow-up period; hence, causes of death were not recorded in the present study.

2.6 | Statistical analysis

Data were analyzed using IBM (Armonk, New York) SPSS Statistics 24 and R version 3.4.0 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

Data are presented as the mean ± SD for continuous variables and as counts with percentages for categorical variables. The significance of differences in patient baseline characteristics for patients alive at end study and patients who died during follow-up was explored using χ^2 tests for categorical data and independent samples *t* tests for continuous data.

Cox regression models were used to estimate all-cause mortality hazard ratio (HR) with 95% confidence intervals (CIs) for HbA1c, FPG, and 2hPG values at baseline. Multivariate Cox regression analysis was performed using HbA1c, FPG, and 2hPG values as continuous variables. A Firth regression model was used to estimate all-cause mortality HRs according to different vascular pathologies and glycemic categories based on both HbA1c and OGTT results. Event-free time was the time from study inclusion until death or censoring. All patients were monitored from enrolment until death or until 30 August 2016. Based on the selection of appropriate covariates according to known risk factors associated with DM and PAD, three models were constructed to evaluate the effect of possible confounding. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, and the use of platelet inhibitors, statins, and antihypertensive medication. Model 3 was a fully adjusted model that included low-density lipoprotein cholesterol, smoking status, a history of coronary artery disease (CAD), a history of cerebrovascular disease and a history of reduced renal function in addition to the covariates included in Models 1 and 2. Model fit was examined by a global test of the proportional hazards assumption. Complete-case analyses were used for all survival models. The variance inflation factor was used to test for multicollinearity.

Kaplan–Meier curves were used to illustrate survival with time according to glycemic status based on OGTT results (FPG and 2hPG values) and HbA1c values. The log-rank test was used to test for differences in survival across glycemic categories.

A power calculation was not performed prior to the recruitment of patients to the study and this study should therefore be considered exploratory. However, the number of events per variable was within an acceptable level.

3 | RESULTS

3.1 | Glycemic status

Baseline characteristics of the study population based on glycemic status are presented in Table 1. The prevalence of newly diagnosed DM was 12% based on OGTT results and 15% based on HbA1c levels (Figure 2; Supporting Information Table S1). The HbA1c values and OGTT results largely classified different patients as having newly diagnosed DM. Twenty-five of 40 patients (63%) with HbA1c ≥6.5% had a non-DM OGTT result. In addition, 22% of patients categorized with prediabetes based on OGTT results had an HbA1c ≥6.5%.

3.2 | Mortality

All-cause mortality was 40% (n = 110) among the 273 vascular surgery patients with unknown glucose status at baseline, and higher for patients with AAA (55%) and infrainguinal occlusive disease (45%) than for those with carotid stenosis (24%) and IOD (15%); (Supporting Information Table S2). Mortality in patients with known DM at baseline (n = 67) was 79%. Patients who died during follow-up had significantly higher mean HbA1c at baseline than patients who were alive at the end of the study. Patients who died during follow-up were also older, had a higher prevalence of reduced renal function, and more often had a history of CAD (Table 2). Patients who were alive at the end of the study were more often treated with antiplatelet therapy than those who died during follow-up. The mean follow-up time was 2805 days and the median follow-up time was 3296 days (range 0-3779 days). Independent variables for

TABLE 1 Baseline characteristics of study participants (n = 273) by glycemic status (follow-up ended on 30 August 2016 = 7.8 years)

		HbA1c (%)		OGTT[b]	
Characteristics	**Reference population[a]**	**6.0-6.4**	**≥6.5**	**PreDM[c]**	**DM[d]**
No. participants	70 (26)	122 (45)	40 (15)	90 (34)	33 (12)
Age (years)					
Mean	67.6	70.3	71.6	71.5*	71.1
Range	49-87	35-88	54-89	48-89	59-88
Sex					
Female	21 (30)	38 (31)	8 (20)	15 (17)	8 (24)
Male	49 (70)	84 (69)	32 (80)	75 (83)	25 (76)
BMI (kg/m^2)					
Mean ± SD	25.2 ± 4.3	25.2 ± 4.4	27.7 ± 4.4*	26.4 ± 4.4	27.0 ± 3.3
Range	16.7-35.8	16.4-38.9	18.5-36.1	17.7-38.9	22.2-32.9
No. of cases with missing data	36	55	13	37	12
Vascular pathology					
Carotid stenosis	11 (16)	17 (14)	8 (20)	10 (11)	6 (18)
AAA	16 (23)	25 (21)	8 (20)	25 (28)	7 (21)
IOD	18 (26)	22 (18)	2 (5)	6 (7)	3 (9)
InOD	23 (33)	57 (47)	21 (53)*	48 (53)*	16 (49)
Peripheral arterial aneurysms	2 (3)	1 (1)	1 (3)	1 (1)	1 (3)
Smoking status					
Non-smoker	8 (11)	24 (20)	6 (15)	10 (11)	9 (27)
Former or current smoker	57 (81)	94 (77)	33 (83)	77 (86)	21 (64)
No. of cases with missing data	3 (7)	4 (3)	1 (3)	3 (3)	3 (9)
Renal function[e]					
Normal	60 (86)	87 (71)	25 (63)	56 (62)	24 (73)
Reduced	10 (14)	34 (28)*	13 (33)*	32 (36)*	9 (27)
No. of cases with missing data	0	1 (1)	2 (5)	2	0
Medical history					
Antihypertensive treatment					
No	21 (30)	26 (21)	4 (10)	14 (16)	6 (18)
Yes	49 (70)	96 (79)	36 (90)*	76 (84)	27 (82)
CVD					
No	55 (79)	99 (81)	28 (70)	71 (79)	23 (70)
Yes	15 (21)	23 (19)	12 (30)	19 (21)	10 (30)
CAD					
No	52 (74)	66 (54)	16 (40)	48 (53)	19 (58)
Yes	18 (26)	56 (46)*	24 (60)**	42 (47)*	14 (42)
Medical treatment					
Statins					
No	9 (13)	16 (13)	2 (5)	13 (14)	0
Yes	61 (87)	106 (87)	38 (95)	77 (86)	33 (100)
Antiplatelet agents					
No	6 (9)	20 (16)	7 (18)	17 (19)	1 (3)
Yes	64 (91)	102 (84)	33 (83)	73 (81)	32 (97)
FPG (mmol/L)					
Mean ± SD	5.2 ± 0.5	5.5 ± 0.6*	6.6 ± 1.3**	5.9 ± 0.5**	7.1 ± 1.4**
Range	4.1-6.0	4.2-7.3	4.8-9.8	4.4-6.7	5.2-10.5
2hPG (mmol/L)					
Mean ± SD	5.4 ± 1.2	7.0 ± 3.4**	10.2 ± 3.5**	8.3 ± 1.9**	13.2 ± 4.7**
Range	2.4-7.6	2.9-31.8	3.8-18.8	2.3-10.9	3.0-31.8
No. of cases with missing data	0	0	2	0	2

TABLE 1 (Continued)

Characteristics	Reference population[a]	HbA1c (%)		OGTT[b]	
		6.0-6.4	≥6.5	PreDM[c]	DM[d]
HbA1c (%)					
Mean ± SD	5.7 ± 0.2	6.2 ± 0.1**	6.8 ± 0.5**	6.1 ± 0.4**	6.5 ± 0.7**
Range	5.1-5.9	6.0-6.4	6.5-8.8	5.0-7.2	5.2-8.8
Total cholesterol (mmol/L)					
Mean ± SD	4.6 ± 1.0	4.6 ± 1.1	4.3 ± 1.0	4.5 ± 1.1	4.4 ± 1.0
Range	2.5-6.9	2.5-7.7	2.3-6.7	2.0-9.1	2.3-6.8
No. of cases with missing data	1	0	0	0	0
LDL-C (mmol/L)					
Mean ± SD	2.8 ± 0.9	2.8 ± 1.0	2.5 ± 0.9	2.7 ± 1.1	2.7 ± 0.9
Range	1.1-5.1	0.9-5.5	1.0-4.7	0.9-7.4	1.1-5.0
No. of cases with missing data	2	1	0	0	0

Abbreviations: AAA, abdominal aortic aneurysm; BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease; InOD, infrainguinal occlusive disease; IOD, iliac occlusive disease; LDL-C, low-density lipoprotein cholesterol.
Unless indicated otherwise, data are given as n (%).
*$P < 0.05$,; **$P < 0.001$ compared with the reference population. The *P*-values given in the table refer to the association between patients grouped in different glycemic categories compared with the reference population and were determined using the Chi-squared test for categorical variables and ANOVA for continuous variables.
[a]The reference population consisted of participants with normal HbA1c, fasting plasma glucose (FPG) and 2-hour post-load glucose (2hPG) values.
[b]The oral glucose tolerance test categories are based on both FPG and 2hPG values.
[c]Prediabetes (PreDM) was defined as FPG <7.0 mmol/L and 2hPG between 7.8 and 11.1 mmol/L, and/or FPG between 6.1 and 7.0 mmol/L with a normal 2hPG.
[d]Diabetes mellitus (DM) was defined as FPG ≥7.0 mmol/L and/or 2hPG ≥11.1 mmol/L.
[e]Renal function was classified as normal if the estimated glomerular filtration rate (eGFR) was >60 mL/min per 1.73 m^2 and as reduced if eGFR was <60 mL/min per 1.73 m^2.

TABLE 2 Baseline characteristics according to alive/dead status at the end of the follow-up period

Characteristics	All patients	Died during follow-up	Alive at end of study	*P*-value
Total (%)	273 (100)	110 (40)	163 (60)	
Age (years)				
Mean	69.6	74.1	66.5	<0.0001[a]
Range	35-89	53-89	35-87	
Sex				0.07[b]
Female	73 (26.7)	23 (21)	50 (31)	
Male	200 (73.3)	87 (79)	113 (69)	
BMI (kg/m^2)				
Mean ± SD	25.7 ± 4.3	24.9 ± 4.6	26.2 ± 4.0	0.05[a]
Range	16.4-38.9	16.4-36.1	18.5-38.9	
No. of cases with missing data	112 (41)	46 (42)	66 (40)	
Vascular pathology				
Carotid stenosis	42 (100)	10 (24)	32 (76)	
AAA	60 (100)	33 (55)	27 (45)	
IOD	47 (100)	7 (15)	40 (85)	
InOD	119 (100)	58 (49)	61 (51)	
Peripheral arterial aneurysms	5 (2)	2 (40)	3 (60)	
Smoking status				NS[a]
Non-smoker	42 (15.5)	20 (18)	22 (14)	
Former or current smoker	219 (80)	87 (79)	132 (81)	
No. of cases with missing data	12 (4.5)	3 (3)	9 (6)	
Renal function[c]				0.007[b]
Normal	199 (73)	71 (65)	127 (78)	
Reduced	71 (26)	39 (36)	33 (20)	
No. of cases with missing data	3	0	3 (2)	
Medical history				
CVD				NS[b]
No	212 (78)	84 (76)	131 (80)	

TABLE 2 (Continued)

Characteristics	All patients	Died during follow-up	Alive at end of study	*P*-value
Yes	61 (22)	26 (24)	32 (20)	
No. of cases with missing data	0	0	0	
CAD				<0.0001[b]
No	180 (66)	50 (46)	113 (69)	
Yes	93 (34)	60 (55)	50 (31)	
No. of cases with missing data	0	0	0	
Medical treatment				
Antihypertensives				0.03[b]
No	142 (52)	17 (16)	43 (26)	
Yes	131 (48)	93 (85)	120 (74)	
No. of cases with missing data	0	0	0	
Statins				0.05[b]
No	30 (11)	17 (16)	13 (8)	
Yes	243 (89)	93 (85)	150 (92)	
Antiplatelet agents				<0.0001[b]
No	37 (14)	28 (26)	9 (6)	
Yes	236 (86)	82 (75)	154 (95)	
FPG (mmol/L)				
Mean ± SD	5.7 ± 0.9	5.8 ± 1.1	5.6 ± 0.7	NS[a]
Range	4.1-10.5	4.2-10.5	4.1-9.4	
2hPG (mmol/L)				
Mean ± SD	7.3 ± 3.3	7.8 ± 3.7	7.0 ± 2.9	0.04[a]
Range	2.3-31.8	2.3-31.8	2.4-18.3	
HbA1c (%)				
Mean ± SD	6.1 ± 0.5	6.2 ± 0.5	6.0 ± 0.4	0.003[a]
Range	5.0-8.8	5.1-8.8	5.0-7.9	
Total cholesterol (mmol/L)				
Mean ± SD	4.5 ± 1.1	4.4 ± 1.0	4.6 ± 1.1	NS[a]
Range	2.0-9.1	2.0-7.5	2.5-9.1	
No. of cases with missing data	1 (0.4)	0	1 (0.6)	
LDL-C (mmol/L)				
Mean ± SD	2.8 ± 1.0	2.7 ± 1.0	2.8 ± 1.1	NS[a]
Range	0.9-7.4	0.9-5.1	1.0-7.4	
No. of cases with missing data	3 (1.1)	0	3 (2)	

Abbreviations: 2hPG, 2-hour post-load glucose; AAA, abdominal aortic aneurysm; BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease; FPG, fasting plasma glucose; InOD, infrainguinal occlusive disease; IOD, iliac occlusive disease; LDL-C, low-density lipoprotein cholesterol.
Note, percentages in the table may not add up to 100 due to rounding.
Unless indicated otherwise, data are given as n (%). The *P*-values given in the table refer comparisons between patients who died during follow-up and those who were alive at the end of the study.
[a] Independent samples *t* test.
[b] Chi-squared test.
[c] Renal function was classified as normal if the estimated glomerular filtration rate (eGFR) was >60 mL/min/1.73 m^2 and as reduced if eGFR was <60 mL/min/1.73 m^2.

mortality were age, male sex, and lack of treatment with platelet inhibitors.

Differences in survival between glycemic categories are shown in Figure 3. Patients diagnosed with DM according to HbA1c values had significantly higher mortality during follow-up than patients with normal HbA1c ($P = 0.015$). Patients diagnosed with intermediate hyperglycemia based on OGTT results, but not fulfilling the criteria for DM, had significantly higher mortality than normoglycemic patients ($P = 0.001$).

In crude analysis, as well as in the age- and sex-adjusted Cox regression model (Model 1), HbA1c was significantly associated with all-cause mortality ($P = 0.01$; Table 3). The association remained statistically significant after adjusting for age, sex, and the use of platelet inhibitors, statins, and antihypertensive medication ($P = 0.04$; Model 2, Table 3). In a fully adjusted Cox regression model, the effect size of HbA1c on all-cause mortality remained largely unchanged, but the association was no longer significant ($P = 0.13$; Model 3, Table 3). Fasting plasma glucose and 2hPG values

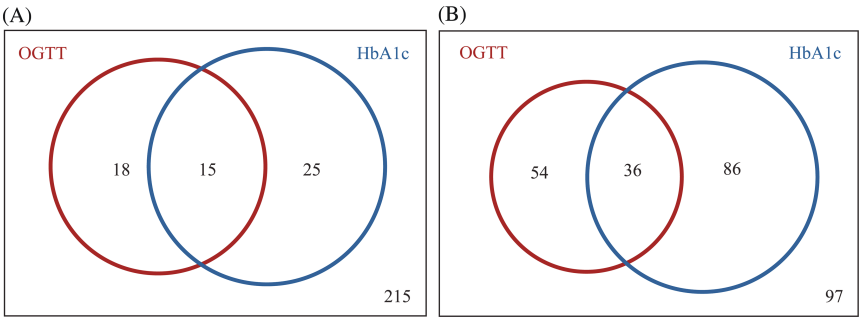

FIGURE 2 Venn diagram showing the prevalence of newly diagnosed A, diabetes mellitus (DM) and B, intermediate hyperglycemia based on HbA1c results (blue circles) and results of the oral glucose tolerance test (OGTT). The two methods largely classified different patients as having newly diagnosed DM and intermediate hyperglycemia: the prevalence of newly diagnosed DM was 12% based on OGTT results and 15% based on HbA1c levels. Twenty-five of 40 patients (63%) with HbA1c ≥6.5% had a non-DM OGTT result

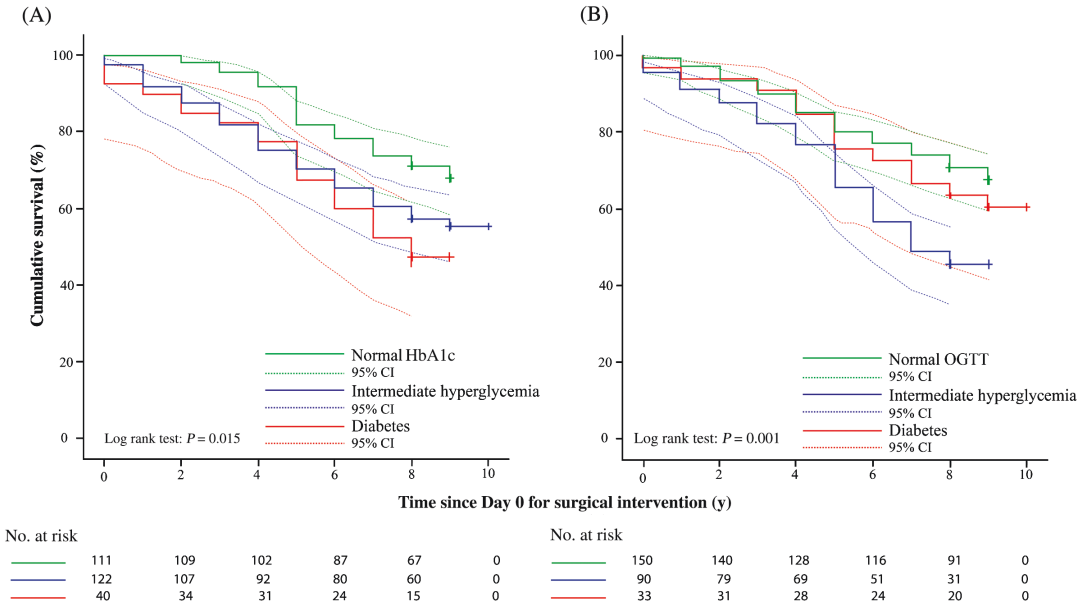

FIGURE 3 Crude survival curves for vascular surgery patients grouped according to A, HbA1c results and B, results of the oral glucose tolerance test (OGTT). Using HbA1c levels, values ≥6.5% (48 mmol/mol) were considered indicative of diabetes mellitus (DM), whereas HbA1c in the range 6.0% to 6.4% (43-46 mmol/mol) was considered intermediate hyperglycemia and HbA1c <6.0% (43 mmol/mol) was considered to indicate normoglycemia. Based on fasting plasma glucose (FPG) values and 2-hour post-glucose load values (2hPG), OGTT results were categorized as follows: DM was defined as FPG ≥ 7.0 mmol/l and/or 2hPG ≥ 11.1 mmol/l; intermediate hyperglycaemia was defined as impaired glucose tolerance (FPG < 7.0 mmol/L and a 2-hour post glucose load value between 7.8 mmol/L and 11.1 mmol/L) and/or impaired fasting glucose (FPG between 6.1 mmol/L and 7.0 mmol/L with a normal 2hPG); and normoglycaemia was defined as FPG <6.1 mmol/L and 2hPG <7.8 mmol/L. CI, confidence interval

were not independent predictors for mortality in any of the three models (Table 3). The proportional hazards assumption was fulfilled for all variables. No variables were found to be highly correlated. The number of events per variable was 110/10 = 11.

Additional analyses of HRs for death according glycemic categories in the different vascular pathologies were performed (Supporting Information Table S3). Results from a fully adjusted Firth regression model were not available for patients with IOD due to a small number of events combined with a high number of adjustment variables.

3.3 | Medical treatment

At the time of inclusion, 243 of 273 patients (89%) were receiving statins, 236 (86%) were receiving antiplatelet

TABLE 3 Hazard ratios for death during follow-up according to HbA1c, fasting plasma glucose and 2-hour post-load glucose values

	HbA1c		FPG		2hPG	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Crude	1.75 (1.24-2.46)	0.01	1.13 (0.93-1.38)	0.21	1.07 (1.01-1.12)	0.01
Model 1	1.67 (1.15-2.44)	0.01	1.12 (0.90-1.40)	0.31	1.03 (0.96-1.09)	0.43
Model 2	1.54 (1.03-2.32)	0.04	1.12 (0.89-1.41)	0.35	1.03 (0.97-1.10)	0.35
Model 3	1.39 (0.92-2.09)	0.13	1.10 (0.87-1.39)	0.44	1.03 (0.96-1.10)	0.42

Abbreviations: CI, confidence interval; FPG, fasting plasma glucose; HR, hazard ratio.
Model 1 was adjusted for age and sex.
Model 2 was adjusted for age, sex, and the use of platelet inhibitors, statins, and antihypertensive medication.
Model 3 was the fully adjusted model, adjusted for low-density lipoprotein cholesterol, smoking status, history of coronary artery disease, history of cerebrovascular disease and history of reduced renal function in addition to the covariates included in Models 1 and 2.

therapy, 22 (8%) were receiving warfarin, and 213 (78%) were receiving antihypertensive treatment. Fifty-four (25%) of the 213 patients who received antihypertensive treatment were being treated with three or more antihypertensives. Nine of the 33 (27%) patients with newly diagnosed DM based on OGTT results received antidiabetic therapy at the end of the study and 13 (39%) died during follow-up. Of the 13 patients who died during follow-up, two patients received medical treatment for DM and 11 did not use antidiabetic medication at study closure.

4 | DISCUSSION

The findings of this study indicate that HbA1c is a useful marker in the preoperative screening of patients of unknown glycemic status admitted for vascular surgery. The results of HbA1c testing may identify people at high risk of long-term mortality following surgical treatment for PAD. Thus, HbA1c results may be of importance for preoperative risk assessment in this group of patients.

To the best of our knowledge, the present study is the only long-term follow-up study to have evaluated HbA1c and OGTT as predictors of mortality in patients with PAD.

Five year all-cause mortality in patients with PAD has been reported to be in the range 19% to 37%,[19–21] and 10-year all-cause mortality has been reported to range from 42% to 54%.[20,22] In accordance with these studies, the total mortality in the present study, when including patients with known DM, was 48%. Research has shown that approximately one-third of patients with type 2 DM are unaware of their DM diagnosis, and are hence untreated and at high risk of developing vascular complications.[23] Similarly, one-third of patients with PAD and DM in the present study were unaware of their DM diagnosis.

Several previous studies have aimed to compare mortality in patients with PAD and with or without established DM. Those studies concluded that individuals with PAD and DM had significantly higher mortality than individuals with PAD only.[1,24–26] In those studies, patients with known DM had been diagnosed using FPG and/or 2hPG values. The studies did not provide information about mortality in patients with newly diagnosed DM. Due to variations in the definition of glycemic status, variations in the vascular pathology studied, and variations in the groups of patients selected for comparison, it is difficult to interpret results from studies investigating mortality and glycemic status in patients with vascular disease. In the present study, patients with established PAD and unknown glycemic status were tested for DM using both HbA1c and OGTT criteria. When categorizing patients as DM, intermediate hyperglycemia, or normoglycemia, patients with newly diagnosed DM based on HbA1c values had significantly higher mortality in crude analysis than patients with normal HbA1c. Results from the OGTTs showed that patients with intermediate hyperglycemia, but not patients with DM, had significantly higher mortality in crude analysis than patients with normal OGTT, as also reported by van Kuijk et al[25] The present study was not designed to explore causality for the association of glycemic status with all-cause mortality. However, several possible explanations may be discussed. Patients with intermediate hyperglycemia based on OGTT results were more likely to be former or current smokers than DM patients. This may have affected the association of OGTT status with all-cause mortality.

Another possible explanation could be the discordance in classification into glycemic categories when using OGTT results compared with HbA1c results. Twenty (22%) of the patients with intermediate hyperglycemia based on OGTT results were categorized as having DM according to HbA1c results.

Patients with DM according to HbA1c and patients with prediabetes based on OGTT results shared some baseline features, which may explain, in part, the similarities in mortality between the two groups. Patients in both categories were significantly older and were more likely to have reduced renal function, a medical history of CAD, and infrainguinal occlusive disease at baseline than participants with normal HbA1c, FPG, and 2hPG values (reference population).

Further, patients with DM based on OGTT results were informed about their DM diagnosis. However, at the time of inclusion in the study, HbA1c was not yet implemented as a diagnostic criterion for DM. Hence, patients with HbA1c ≥6.5% and a non-DM OGTT result were not informed of

their results. Consequently, treatment for newly diagnosed DM may vary between these groups, which may represent a confounding factor in this study. Conversely, only nine patients (27%) with newly diagnosed DM based on OGTT results, of whom five also had DM according to HbA1c values, received medical treatment for DM at the end of the study, reducing the difference in treatment between the groups. Mean HbA1c values in patients with DM categorized based on OGTT results was 6.5%, which is below the recommended target value of HbA1c in the treatment of DM. This may be a possible explanation for the low treatment rate of newly diagnosed DM in this study population.

It has been reported that HbA1c and the OGTT largely define different groups of patients as having DM.[11–13,23] This is in accordance with previous published results from the present study population.[16] Oral glucose tolerance test and HbA1c values represent different metabolic expressions. The OGTT is a stress test of pancreatic islet cell functioning, whereas HbA1c represents long-term exposure to plasma glucose. Sustained hyperglycemia may induce non-enzymatic glycation of lipoproteins and thereby start a cascade of changes in the endothelial cells that may lead to endothelial dysfunction.[27] Thus, HbA1c may, to a greater extent than OGTT results, express the degree of macro- and microvascular inflammation. This could possibly explain the association between HbA1c and all-cause mortality in the present study. However, the present study was not designed to provide an answer to this assumption. Further studies on the association between HbA1c and inflammation are needed to explore whether this association could be a possible target in future research regarding treatment of patients with PAD and DM.

Studies have shown that both OGTT (FPG and 2hPG) and HbA1c levels are associated with future risk of developing DM.[11,28] Of note is the high number of patients categorized as having prediabetes according to HbA1c results in the present study (Figure 2). This study does not provide data on the development from prediabetes into DM during the follow-up period. However, one could speculate that a higher number of patients in the prediabetes group based on HbA1c results may develop DM during the follow-up period than in the prediabetes group based on OGTT results. If so, this would support the conclusion on the association between HbA1c and long-term mortality in the present study. In contrast, recent research has shown that the conversion rate from prediabetes to DM in patients with prediabetes is higher based on OGTT than HbA1c results.[29] Hence, we cannot exclude the possibility that the association between OGTT results and mortality in the present study may be underestimated.

In the present study, HbA1c was a predictor for all-cause mortality in crude analysis, as well as after adjustment for age, sex, and medical treatment. The association of HbA1c with all-cause mortality did not achieve statistical significance in multivariate analysis, although the effect estimation of HbA1c on all-cause mortality remained primarily unchanged. This could be explained by a lack of statistical power due to sample size, and by the possibility of multicollinearity. Hence, this study should be considered an exploratory study. The results from subanalyses of HRs for death according to glycemic categories in patients with different vascular pathologies indicate that the association of HbA1c with all-cause mortality remained significant for patients with carotid stenosis and AAA. No statistically significant association of HRs for death with different glycemic categories was seen for patients with peripheral occlusive disease, although the effect estimation was 1.45 in Model 2. However, the results are affected by a low statistical power in this subanalysis. A larger study is needed to enable solid conclusions to be reached for each of the vascular pathologies.

O'Sullivan et al[30] investigated 30-day morbidity and mortality, as well as 6-month mortality, in 122 PAD patients without DM and found a significant association between HbA1c and 30-day morbidity. In contrast with the findings of the present study, O'Sullivan et al[30] did not find an association of HbA1c with mortality. Fifty-eight percent of patients without DM had HbA1c values of 6.0% to 6.9%, and nine patients died during a follow-up period of 6 months.[30] The results of O'Sullivan et al[30] were affected by a small sample size, a short follow-up time, and selection bias regarding glycemic status.

In accordance with the results of the present study, data on general populations in both Western countries and Asian populations have demonstrated a positive association of HbA1c levels with morbidity and mortality of cardiovascular disease in people without DM.[31–33]

Due to an established outpatient program at the Department of Vascular Surgery, Haukeland University Hospital, no patients were lost during follow-up. However, the fact that 66 patients declined to participate in the study and a further 21 patients died before deciding to participate may have introduced a selection bias. Of the 66 who declined to take part in the study, 50% were male. Therefore, a selection bias regarding sex cannot be excluded. However, mean FPG values and the prevalence of newly diagnosed DM based on FPG values alone were the same in the 66 patients as in the study participants. The 21 patients who died had high morbidity and were older than the study population, and are therefore not necessarily comparable to the participants in the present study. Thus, selection bias is believed to have had only a minor effect on overall conclusions, although it cannot be excluded.

Finally, an autopsy was only performed on one-third of patients who died during the follow-up period. Hence, only all-cause mortality was recorded in the present study.

5 | CONCLUSION

The present exploratory study suggests that HbA1c is a useful marker in the preoperative screening of PAD patients

with unknown glycemic status at the time of admission for vascular surgery. The HbA1c results may identify people at high risk of long-term mortality following surgical treatment for PAD. Clinicians should consider including HbA1c for preoperative risk assessment in PAD patients.

A large-scale study should be performed to confirm the results reported herein and to evaluate whether the conclusion holds for different vascular pathologies. Further, an intervention study targeting glucose control should be considered to explore possible implications on mortality and relapse of the vascular pathology.

In addition, the possible association of HbA1c with hyperinsulinemia and inflammatory markers in patients with PAD should be investigated as a potential target in the treatment of patients with PAD and abnormal glucose metabolism.

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DISCLOSURE

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary Table 1. The number of patients categorized as having normoglycaemia, prediabetes and diabetes mellitus according to HbA_{1c}[a] versus OGTT[b] results.

		HbA_{1c}[a]		
OGTT[b]	Subjects	Normo-glycaemia (%)	Prediabetes (%)	Diabetes mellitus (%)
Total	273	111 (40.7)	122 (44.7)	40 (14.7)
Normoglycaemia	150	70 (46.7)	75 (50.0)	5 (3.3)
Prediabetes	90	34 (37.8)	36 (40.0)	20 (22.2)
Diabetes mellitus	33	7 (21.2)	11 (33.3)	15 (45.5)

[a] $HbA_{1c} \geq 6.5\%$ (48 mmol/mol) = diabetes mellitus, HbA_{1c} range of 6.0-6.4% (42-46 mmol/mol) = prediabetes, and $HbA_{1c} < 6.0\%$ (< 42 mmol/mol) = normal HbA_{1c}.

[b] FPG+2-h post glucose load value.

Supplementary Table 2. Mortality according to affected vascular bed.

Vascular pathology	Alive at end study *n* (%)	Dead during follow-up *n* (%)	Total
Carotid stenosis	32 (76)	10 (24)	42 (15)
AAA*	27 (45)	33 (55)	60 (22)
IOD**	40 (85)	7 (15)	47 (17)
Infrainguinal occlusive disease	61 (51)	58 (49)	119 (44)
Peripheral aneurysmal disease	3 (60)	2 (40)	5 (2)
Total	163 (60)	110 (40)	273 (100)

* AAA=abdominal aortic aneurysm

**IOD=inguinal occlusive disease

Supplementary Table 3. HR for death in glycaemic categories and according to affected vascular bed.

	HbA_{1c}			FPG			2-h value		
Vascular pathology	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Model 1[a]									
Overall[d]	1.67	1.15-2.44	0.01	1.12	0.90-1.40	0.31	1.03	0.96-1.09	0.43
Carotid stenosis[e]	9.79	1.36-97.3	0.02	1.52	0.63-3.05	0.31	1.01	0.80-1.21	0.92
AAA[e]	2.17	0.99-4.34	0.05	1.21	0.80-1.24	0.24	1.08	0.96-1.21	0.18
IOD[e]	0.31	0.02-2.70	0.31	0.16	0.02-0.74	0.02	0.57	0.31-0.96	0.03
Peripheral occlusive[e]	1.46	0.85-2.33	0.16	1.09	0.81-1.41	0.55	1.04	0.96-1.12	0.34
Model 2[b]									
Overall[d]	1.54	1.03-2.32	0.04	1.12	0.89-1.41	0.35	1.03	0.97-1.10	0.35
Carotid stenosis[e]	10.9	1.21-141	0.03	1.45	0.55-3.03	0.41	1.00	0.77-1.22	0.99
AAA[e]	2.53	1.05-5.44	0.04	1.20	0.79-1.73	0.37	1.08	0.94-1.23	0.27
IOD[e]	0.23	0.02-2.42	0.24	0.16	0.02-0.85	0.00	0.38	0.15-0.82	0.01
Peripheral occlusive[e]	1.45	0.84-2.34	0.17	1.12	0.82-1.46	0.44	1.04	0.95-1.13	0.34
Model 3[c]									
Overall[d]	1.39	0.92-2.09	0.13	1.10	0.87-1.39	0.44	1.03	0.96-1.10	0.42
Carotid stenosis[e]	69.2	2.7-14675	0.01	1.39	0.52-3.14	0.48	1.11	0.80-1.40	0.46
AAA[e]	2.93	1.08-7.02	0.04	1.07	0.69-1.57	0.74	1.09	0.94-1.24	0.24
IOD[e]	1.00	na	1.00	1.00	na	1.00	1.0	na	1.00
Peripheral occlusive[e]	1.14	0.66-1.90	0.63	1.00	0.73-1.33	0.98	1.03	0.95-1.11	0.49

[a] Adjusted for age and sex.

[b] Adjusted for age, sex, and the use of platelet inhibitors, statins and antihypertensive medication.

[c] Fully adjusted model. Adjusted for low-density lipoprotein cholesterol, smoking status, history of coronary artery disease, history of cerebrovascular disease and history of reduced renal function in addition to covariates included in Models 1 and 2.

[d] estimation by Cox regression model.

[e] estimation by Firth regression model.

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