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Sistema Sanitario Regione Liguria
Istituto di Ricovero e Cura a Carattere Scientifico

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CICLO XXXVI

Leveraging Biobank to Reveal Novel Pathophysiological Insights and Clinical Correlates in Abdominal Aortic Aneurysm Research.

Relatore:
Prof. Giovanni Pratesi

Candidato:
Dott. Jorge Miguel Mena Vera

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Abstract

Background: Investigating Abdominal Aortic Aneurysms (AAA) requires an intricate understanding of their underlying pathophysiology. In this context, the Genoa Tissue Bank - Vascular Division (GTB-VD) biobank's role becomes pivotal, offering a nuanced approach to research by providing well-curated and extensive biological data. This study utilizes these resources to explore the complex molecular mechanisms and clinical aspects of AAA.

Methods: Employing the GTB-VD biobank's comprehensive repository, our research involved analysis of biological specimens from patients undergoing surgery for AAA and carotid artery stenosis. We focused on evaluating the Nrf2/AhR and HO-1 pathways using techniques such as real-time PCR and ELISA, integrated with patient clinical data. The study's design aimed to utilize the rich diversity of biobank data to glean insights into AAA while showcasing the impact of effective biobank management on enhancing research quality.

Results: The investigation revealed differential protein expression of AhR and HO-1 in AAA patients compared to those with carotid stenosis, despite no significant differences at the mRNA level in PBMCs. Serum HO-1 levels varied between the two groups, with a median level statistically higher in carotid stenosis patients. Furthermore, the relationship between serum HO-1 levels and factors like age, renal function, and erythrocyte count was explored, showing only weak correlations. The GTB-VD biobank has facilitated a detailed exploration of these molecular aspects, showcasing the importance of systematic biobanking in vascular disease research.

Conclusion: This study underscores the intricate molecular pathways involved in AAA and highlights the value of a well-structured biobank in enriching vascular disease research. It infers the involvement of Nrf2/AhR and HO-1 pathways in the pathogenesis of AAA and highlights serum HO-1's potential as a biomarker. By tapping into the GTB-VD biobank's extensive and diverse data, we could illuminate various aspects of AAA pathophysiology, demonstrating how organized biobanking can significantly contribute to complex disease research. Future endeavors should continue leveraging such resources, incorporating broader patient cohorts and diverse analytical methods, to further advance the understanding of AAA and similar vascular conditions.

Keywords: Abdominal Aortic Aneurysms, Biobanking, Nrf2/AhR Pathway, HO-1 Pathway, Vascular Disease Research, Clinical Data Integration, Molecular Biomarkers.

Introduction

Abdominal Aortic Aneurysms: An Overview

Abdominal aortic aneurysms (AAA) refer to the lasting enlargement of a specific segment of the abdominal aorta, typically measuring ≥ 3.0 cm in the infrarenal region, resulting from a weakening of the aortic wall. In most instances, individuals with AAA remain asymptomatic; however, once an aneurysm ruptures, it can prove fatal in up to 80% of cases. Risk factor modeling suggests that there may be over one million individuals in the United States affected by AAA, with approximately 25,000 reported deaths annually attributed to aneurysm ruptures[1].

For more than four decades, the primary approaches to treating aortic aneurysms have involved open surgical repair and the replacement of the affected aortic segment with a synthetic graft. However, in the past decade, endovascular aneurysm repair (EVAR) has emerged as the predominant treatment option due to its significant advantages in terms of reduced mortality rates and shorter hospital stays compared to open surgical repair[2].

The epidemiology AAA formation appears to exhibit notable differences from that of atherosclerotic disease. One of the early and comprehensive studies that examined risk factors for AAA was conducted by the Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. This retrospective analysis yielded significant findings, highlighting the pivotal role of certain factors in AAA development. Specifically, the study identified a history of cigarette smoking as the most potent risk factor for AAA, with a relative risk of 5.9 when compared to non-smokers. Following closely was an age-independent family history of AAA, which carried a relative risk of 1.9, signifying its importance as a risk factor. In contrast, hypertension, hypercholesterolemia, and pre-existing coronary artery disease exhibited relative risks of less than 1.5, implying that the mechanisms underlying AAA may diverge from those associated with atherosclerosis[3].

Despite efforts made, by our and other research groups, in understanding the determinants and pathophysiological mechanisms of AAA (onset, progression, and complications), a lot remains to be unraveled. Nonetheless, some evidence is emerging from the literature on the different processes that could potentially lead or predispose AAA.

The pathophysiological mechanisms governing the formation and progression of AAA are complex, involving numerous signaling cascades and risk factors[1]. Consequently, there is no singular mechanism that can be pinpointed as the sole contributor to this pathology[4]. Nevertheless, there is a consensus among researchers that inflammation of the aortic wall, the reduction of medial smooth muscle cells (SMCs), and the degradation of the extracellular matrix (ECM) are the principal molecular processes involved[4].

The Role of Nrf2/AhR and HO-1 Pathways in AAA Development

In AAA, displaying specific inflammasome fingerprints, several cellular actors have been described to be involved in pathways that could potentially lead to arterial wall weakening, cells dysfunction, and extracellular matrix degradation: Nrf2 (Nuclear factor erythroid 2-related factor 2); AhR (Aryl hydrocarbon receptor); HO-1 (Heme oxygenase 1)[5].

In particular, deregulation of these factors as well as their interaction have been associated with inflammasome activation restraint[6–8], vascular aging[9], and inflammatory/pro-fibrotic factors induction[10]. Thus, further study of the related pathways of these factors could lead to the identification of potential biomarkers of the AAA disease.

Nrf2 plays a crucial role in regulating the transcription of genes responsible for antioxidant and detoxifying functions, including enzymes involved in glutathione (GSH) synthesis, NQO1, and HO-1[11]. Recent research using rodent models has underscored the significance of Nrf2 activity in preventing AAA development. In particular the absence of a transcriptionally active form of Nrf2 in mice has been associated with an elevated risk of developing and experiencing ruptures in AAA. This heightened susceptibility appears to be linked, at least in part, to vascular damage stemming from an accelerated turnover of collagen and an augmented inflammatory response[12].

HO-1, in particular, holds a pivotal role in responding to stressors by generating metabolites like carbon monoxide and bilirubin, which contribute to vascular protection[13]. Studies in rodent models have shown that HO-1 haploinsufficiency can facilitate the induction of AAA[14], while complete loss of HO-1 exacerbates the incidence and rupture of AAAs[15].

In human patients with AAA, there is an observed higher frequency of specific polymorphisms on the HO-1 promoter, limiting HO-1 induction, which suggests a potential role in AAA development[16]. However, the role of HO-1 in humans remains contentious, as recent findings have indicated an upregulation of HO-1 in specimens from enlarged AAAs[17]. Despite the proposal of HO-1 serum levels as a potential biomarker for other cardiovascular diseases[18], there is currently no conclusive evidence linking HO-1 serum levels to AAA in humans.

The aryl hydrocarbon receptor (AhR) encodes detoxifying genes and enhances antioxidant defenses. Both AhR and the Nrf2/HO-1 pathway are involved in restraining inflammasome activation, a contributor to AAA development through the degradation of contractile proteins[6–8]. However, AhR overstimulation has been correlated with vascular aging and the induction of catabolic factors[9,10,19].

Therefore, there is a need for further investigation into the connections between AhR, its family member BMAL1 (Brain and Muscle AhR nuclear translocator-like protein-1), which plays a role in regulating Nrf2-dependent cellular responses[20], and the Nrf2/HO-1 pathways. This exploration should encompass different cell types within the vascular wall and various stages of AAA progression, both in isolation and in relation to metabolic and inflammatory signaling pathways.

Biobanking: Unlocking the Power of Biological Data

In the past two decades, significant advancements in the realm of biological knowledge and patient care have been greatly facilitated by the emergence of -omics sciences, including genomics, transcriptomics, proteomics, and metabolomics, as well as the field of Systems Medicine. Systems Medicine is characterized as an integrative approach to addressing medical needs, drawing upon a wide array of scientific disciplines. Its primary goal is to improve risk prediction and individualized treatment, all while upholding ethical and legal standards. These innovative disciplines require a high level of quality, traceability of biospecimens, and standardized data collection to ensure the production of comparable data and the integration of information for analysis.

To meet these demanding requirements, biobanks have evolved from simple repositories of biological samples into intricate and dynamic entities that are seamlessly integrated into multi-organizational infrastructure networks. Academic Medical Centers are exceptionally well-suited for establishing and managing biobanks, given their capacity to provide access to human subjects, biological specimens, and clinical data. Moreover, biobanking activities align with their mission of education, research, and patient care[21].

Biobanks can be categorized as either population-based, encompassing epidemiological data collected from patients or volunteers without specific inclusion or exclusion criteria, or 'disease-oriented,' focusing on specific populations with particular diseases, as is commonly observed in those dedicated to the study of oncology and cardiovascular conditions.

Approximately 15 years ago, our research unit embarked on this journey by assembling biological specimens and clinical-demographic data from diverse patient cohorts afflicted with vascular diseases such as varicose veins and artery diseases. This ongoing endeavor has fostered collaborative research initiatives with both institutional and national as well as international research teams.

In particular, we have gathered biological specimens and data from individuals undergoing elective surgeries for carotid artery stenosis and AAA. The substantial number of these surgical interventions is driven by the imperative need to mitigate the potentially catastrophic consequences of ischemic stroke, resulting from the vulnerability of carotid plaques, as well as the threat of abdominal aortic rupture. These interventions are based on a statistical risk assessment derived from morphometric parameters observed through arterial imaging.

The scarcity of knowledge pertaining to the underlying biological mechanisms responsible for these significant adverse events, the absence of specific biomarkers for risk assessment, and the dearth of therapeutic targets prompted us to establish the Genoa Vascular Biobank in 2018. This biobank focuses on enrolling patients with carotid artery stenosis and AAA. The recruitment process involves a systematic selection of individuals based on predetermined inclusion and exclusion criteria. Biological specimens are meticulously collected under controlled conditions, and personal data are meticulously organized.

The aim of this project is to investigate Nrf2/HO-1 pathways cell pathways for their participation in AAA development and progression, in order to detect new histological target and systemic biomarkers to speed up the identification of pharmacological intervention and to improve the AAA care plan and management.

Samples for the study will be obtained from the Genoa Tissue Bank-Vascular Division (GTB-VD). Therefore, the first part of this project consists in improving the management of the biobank, including the clinical data associated with the patients enrolled in the biobank.

In this context, our goal is to provide an overview of the network, workflow, ethical considerations, and legal aspects of the GTB-VD, along with a summary of the resources accumulated to date.

Material and Methods

GTB Biobanking

The GTB-VD initiative comprises various crucial components, including the Vascular and Endovascular Surgery Unit, the Laboratory of Experimental and Clinical Vascular Biology (referred to as BioVasc Lab), and the Anatomic Pathology Unit within the Department of Surgical and Diagnostic Sciences at the University of Genoa. Additionally, the management and coordination of storage facilities are overseen by the Biological Resource Center (CRB-HSM) at the IRCCS Ospedale Policlinico San Martino.

To ensure the proper handling of biospecimens, the hospital transport team is responsible for promptly delivering specimens, maintaining them at room temperature, within two hours of collection from the Unit of Vascular and Endovascular Surgery to the BioVasc Lab.

The workflow involves for main steps:

1. Selection and Recruitment of Participants and Biospecimen Collection:

Patients admitted for elective surgery to address carotid stenosis or AAA are considered for inclusion in the biobank. Eligible patients are identified during admission to the Vascular and Endovascular Surgery Unit one day prior to the intervention. They are offered the opportunity to participate in the Biobank and undergo the informed consent process. Blood and urine samples are collected from participants one day before surgery, while tissue samples are obtained during open repair procedures.

2. Reception and Processing of Samples:

Upon the arrival of the samples at the BioVasc Lab, operators assess the quality of the samples, considering factors such as the time elapsed between collection and delivery and the presence of hemolysis in plasma or serum. An alphanumeric code is electronically assigned to each patient using an informatics tool. This code ensures the proper association of specimens with clinical and demographic data while maintaining patient confidentiality. The code is then recorded on labels for cryovials containing frozen specimens and jars with 10% formalin for tissue biopsies. Samples that do not meet the criteria for biobanking are disposed of after informing the patient, who is required to sign a specific form. Blood, urine, and tissue samples are processed in a dedicated room under a sterile laminar flow hood, as detailed in the "Biospecimen collection and processing" paragraph.

3. Storage:

Frozen tissue samples are securely stored in monitored and alarmed facilities at -80°C, under the management of CRB-HSM. Formalin-fixed, paraffin-embedded (FFPE) samples are stored in specialized histoteques within the Anatomic Pathology Unit.

4. Data Collection:

Clinical data collections are integrated into the prospective electronic database system of the Italian Society of Vascular and Endovascular Surgery (https://www.sicvereg.it/).

Ethics and Governance of Genoa Tissue Bank - VD

The Vascular Bank Division is part of the Clinical Pathology Tissue Bank (Genoa Tissue Bank - GTB) at IRCCS Ospedale Policlinico San Martino, affiliated with the Institute's Biological Resources Center (CRB-HSM), together with the Neurological Bank and the Cell Bank.

Samples and data stored in the CRB-HSM can be accessed by the scientific community through a transparent access procedure that aligns with the guidelines of the General Data Protection Regulation (GDPR) - Regulation (EU) 2016/679 of the European Parliament and the Council dated 27th April 2016, which focuses on safeguarding individuals' personal data and facilitating its free movement. This procedure also adheres to the general access conditions set forth by BBMRI ERIC, a consortium of biobanks that includes the CRB-HSM.

Access to samples and data upholds the commitments made to donors to adheres to the principles of equitable access and scientific excellence. It is exclusively granted for specific research projects based on the participant's consent terms, subject to approval by both the institutional Research Ethics Committee and an internal CRB-HSM Evaluation Committee.

Biological sample collection for research purposes begins only after obtaining written informed consent from participants, following comprehensive information provision in accordance with privacy regulations. Consent is obtained prior to blood withdrawal and surgery, and the documented informed consent becomes part of the patient's medical record. A securely archived copy of the consent, in both paper and/or electronic format, is accessible solely to authorized personnel and is managed in compliance with current personal data legislation. Consent, characterized as authentic, voluntary, autonomous, informed, and responsible, can be revoked without any adverse consequences for the participant. In case of revocation, all data in CRB-HSM will be erased, and the biological sample will either be destroyed, anonymized, or made available to the participant for collection.

Collection of Peripheral Blood and Urine Samples

Fasting peripheral blood samples are obtained from the antecubital vein and placed into three tubes, each with a maximum capacity of 6 mL (Vacuette Greiner Bio-One International GmbH). One of these tubes contains a clot activator and gel separator, specifically designed for serum collection, while the other two tubes contain K3-EDTA for plasma, whole blood, and Peripheral Blood Mononuclear Cells (PBMCs) isolation.

Plasma and sera are separated through centrifugation at 3500 × g for 15 minutes at room temperature, and subsequently stored in 500 µL aliquots. Whole blood is preserved in 250 µL aliquots. For the isolation of PBMCs, 6 mL of blood is initially diluted with an equal volume of saline

(1:1 ratio) in 15 mL tubes. Density gradient centrifugation is then conducted over 3 mL of Lympholyte separation medium (Cedarlane, Canada) at 1800 × g for 20 minutes without braking. The interface ring, containing PBMCs, is meticulously recovered, rinsed with saline through centrifugation, and the resulting pellet is divided into four aliquots. These aliquots undergo a brief high-speed centrifugation (30 seconds) before being dry stored at -80°C. Urine samples are exclusively collected from patients diagnosed with AAA and are carefully preserved in six vials, each containing 1 mL of urine. These vials are stored within monitored and alarmed facilities at -80°C.

Collection of Tissue Samples

Following surgical excision, tissue samples are placed in sterile, disposable containers and transported from the operating room to the BioVasc Lab. The tissue is processed in consecutive segments based on size and quality. Typically, two aliquots, each weighing approximately 0.5 to 1 gram, are promptly stored at -80°C for subsequent molecular analysis. The remaining tissue segments are fixed in 10% formalin at room temperature and then sent to the Anatomy Pathology Unit. Here, they follow standard protocols, beginning with immersion in 10% buffered formalin for an overnight period (approximately 12-18 hours). Subsequently, the tissue undergoes routine processing and embedding in paraffin to produce histologic slides measuring 3-5 µm in thickness, which are later stained with hematoxylin/eosin.

Upon completion of the standard diagnostic procedure, the paraffin blocks are meticulously archived at room temperature, stored in cardboard boxes shielded from dust, light, and heat sources.

Quality Control Testing for Biological Specimens

When necessary, we evaluate the RNA integrity number (RIN) of dry-frozen PBMCs using the Agilent 2100 Bioanalyzer in conjunction with the RNA 6000 Nano Kit (Agilent Technologies, Waldbronn, Germany). This assessment is conducted strictly in accordance with the manufacturer's provided instructions.

For all Formalin-Fixed Paraffin-Embedded (FFPE) segments, we prepare consecutive sections with a thickness ranging from 3-5 µm. These sections undergo staining with both hematoxylin-eosin (HE) and MOVAT to assess tissue morphology.

Collection and Management of Clinical Data

Each sample housed within the GTB-VD is linked to clinical data corresponding to the respective donor patient. Until 2021, the method of collecting clinical data involved storing physical copies of patient clinical records. Starting in January 2021, clinical data is now collected using the prospective electronic database system provided by the Italian Society of Vascular and Endovascular Surgery (https://www.sicvereg.it/). Clinicians enter all available patient data into this electronic database system, facilitating the computation and analysis of clinical data associated with the biobank samples.

The electronic database system does not contain patient names or any identifiable patient information. Patients are registered using a study number that is automatically assigned by a designated tool within the system. This unique study number is used throughout the data collection and processing phases, ensuring patient anonymity and data security.

cDNA Reverse Transcription and Quantitative Reverse Transcription-PCR

For total RNA extraction, TRIZOL reagent (Invitrogen) was employed following the manufacturer's instructions. Subsequently, 1 µg of RNA was reverse-transcribed into cDNA utilizing random hexamer primers and SuperScript II Reverse Transcriptase (Invitrogen). The gene expression analysis for NFE2L2 (Nrf2), HMOX1 (HO1), AhR, AhRR, and RPLP0 was conducted using pre-designed primers from PrimeTime Mini qPCR Assay by IDT TemaRicerca (Italy). This analysis was performed on a 96 light cycler Real Time PCR System (Roche) with the Luna Universal qPCR Master Mix (EuroClone, Italy). Normalization was carried out using b-actin expression levels. Normalization was carried out using b-actin expression levels. Each assay was conducted in triplicate, and the 2-ΔΔCT method was employed for relative quantification of mRNA fold induction.

ELISA assays

ELISA assays were conducted using 96-well Maxi Sorp Nunc plates, with each well receiving 0.5 µg/100 µl of the prepared MDA-BSA adduct. To prevent non-specific binding, a solution containing 1% BSA in 10 mM PBS at pH 7.4 was applied. Subsequently, anti-HO1 antibodies (ab207621-1x96 test Human Heme Oxygenase 1 -Kit Elisa) were introduced. As a secondary antibody, an anti-rabbit antibody labeled with peroxidase (at a concentration of 1:1000) was utilized. Detection was achieved using ortho-phenylenediamine (OPD) and H_2O_2, followed by development with 5% sulfuric acid in water. Readings were taken at 490 nm using a multi-well plate reader.

Statistical analysis

All statistical analyses were conducted utilizing R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). A significance level of $p < 0.05$ was employed to determine statistical significance. The presentation of data was tailored to the nature of the variables. For normally distributed data, the mean and standard deviation (SD) were provided, while non-normally distributed data were presented as the median [IQR] (Interquartile Range). To compare Gaussian-distributed data, an unpaired t-test was employed, whereas non-Gaussian-distributed data were assessed using the Mann–Whitney U test. Categorical data were analyzed utilizing the $\chi 2$ test or Fisher's exact test, as appropriate for the specific circumstances.

Results

Clinical database and Biobanking

The GTB-VD functions as a prospective observational biobank and databank, with a specific emphasis on patients afflicted with AAA and carotid artery diseases. Since November 2018, the GTB-VD has been collecting biological samples obtained from patients who have undergone surgical procedures for carotid stenosis and AAA at the Vascular and Endovascular Surgery Unit of IRCCS Ospedale Policlinico San Martino in Genoa, Italy. As of now, a total of 537 subjects have been enrolled in the biobank. ***Table 1*** offers an overview of the number of cases for which collection of biological samples is accessible.

Table 1 | Number of patients enrolled in the biobank from November 2018 to July 2023.

Type of Surgical Procedure	N. Patients
CAS	40
CEA	311
EVAR	117
OSR	69
Total	**537**

CAS, carotid stenting; CEA, carotid endarterectomy, EVAR, endovascular repair; OSR, open surgical repair

In the biobank, various types of biological samples are systematically collected, including peripheral blood (consisting of serum, plasma, whole blood, and PBMCs), as well as plaque tissue when open surgery is conducted. Additionally, for patients diagnosed with AAA, urine samples are also meticulously collected and preserved. (***Table 2***).

Table 2 | Number of samples collected in the biobank for each biological sample.

Surgical Procedure	*N. Tissue cryovials*	*N. Whole blood cryovials*	*N. Serum cryovials*	*N. Plasma cryovials*	*N. PBMC cryovials*	*N. Urine cryovials*
CAS	0	145	185	186	125	0
CEA	468	642	1024	982	571	0
EVAR	0	443	504	528	403	646
OSR	101	121	221	194	106	183
Total	**569**	**1351**	**1934**	**1890**	**1205**	**829**

CAS, carotid stenting; CEA, carotid endarterectomy, EVAR, endovascular repair; OSR, open surgical repair

Each specimen preserved within the GTB-VD is linked to clinical data corresponding to the respective donor patient. Prior to 2021, the method for collecting clinical data involved archiving physical copies of patient clinical records. However, commencing in January 2021, clinical data is now systematically collected using the prospective electronic database system provided by the Italian Society of Vascular and Endovascular Surgery (https://www.sicvereg.it/). Healthcare professionals diligently record patient data within this electronic database system, enabling computation and analysis of clinical data associated with the biobank samples.

It is essential to note that the electronic database system does not contain patient names or any identifiable patient information. Patients are registered using a study number that is electronically assigned by a designated tool. This study number is utilized throughout the data collection and processing stages, ensuring patient anonymity and safeguarding sensitive information.

The primary objective of the SICVE registry is to amass clinical data from all patients who have undergone surgical interventions for carotid artery stenosis, peripheral artery stenosis, and abdominal aortic aneurysms at various hospitals across Italy that have chosen to participate in the registry.

The database system was introduced in January 2021, with data collection commencing in February. An export procedure was established to enable each hospital to download all the clinical data they have inputted. This facilitated quality control checks to ensure the completeness and reliability of the recorded data thus far.

Preliminary data from the SICVEREG clinical database

The following section will provide an illustrative example of the data that is being collected in the SICVE registry. Specifically, this example pertains to data collected for the surgical procedures conducted in 2021. The aim is to showcase the nature of the data encompassed within the registry and elucidate the quality control process implemented. The data undergoes a systematic and iterative quality control procedure, designed to guarantee its completeness and accuracy.

The data collection in the SICVE registry is divided in 5 sections (anamnesis, intraoperative, postoperative, follow up at 30 days, follow up at over 30 days). **Table 3** and **Table 4** show a summary of the anamnestic section for the two pathologies of interest included in the biobank, carotid stenosis, and AAA respectively.

Table 3 | Clinical history and anamnestic details of patients who underwent surgery for carotid stenosis.

Variable		Carotid stenosis (n = 143)
Indication (%)	Amaurosis	8 (5.6)
	Asymptomatic	109 (76.2)
	Cerebral TIA	11 (7.7)
	Minor Stroke (NIHSS 1-5)	10 (7.0)
	Moderate Stroke (NIHSS 6-15)	4 (2.8)
	NA	1 (0.7)
Sex (%)	Female	41 (28.7)
	Male	101 (70.6)
	NA	1 (0.7)
Hypertension History (%)	NO	27 (18.9)
	YES	115 (80.4)
	NA	1 (0.7)
Diabetes (%)	NO	94 (65.7)
	YES	45 (31.5)
	NA	4 (2.8)
IRC (%)	NO	105 (73.4)
	YES	30 (21.0)
	NA	8 (5.6)
Smoke (%)	NO	99 (69.2)
	YES	42 (29.4)
	NA	2 (1.4)
Dyslipidemia (%)	NO	46 (32.2)
	YES	95 (66.4)
	NA	2 (1.4)
Cardiac History (%)	NO	106 (74.1)
	NYHA 1	9 (6.3)
	NYHA 2	23 (16.1)
	NYHA 3	4 (2.8)

	NA	1 (0.7)
Ischemic Heart Disease (%)	NO	91 (63.6)
	YES	34 (23.8)
	NA	18 (12.6)
Pulmonary History (%)	NO	74 (51.7)
	YES	66 (46.2)
	NA	3 (2.1)
Stroke History (%)	Contralateral	5 (3.5)
	Ipsilateral	3 (2.1)
	NO	134 (93.7)
	NA	1 (0.7)
Preop Thrombolysis (%)	NO	127 (88.8)
	YES	6 (4.2)
	NA	10 (7.0)
Grade Stenosis (%)	0 - 49 %	2 (1.4)
	50 - 69 %	14 (9.8)
	70 - 99 %	123 (86.0)
	Occluded	3 (2.1)
	NA	1 (0.7)
Contralateral Stenosis Grade (%)	0 - 49 %	107 (74.8)
	50 - 69 %	29 (20.3)
	70 - 99 %	5 (3.5)
	Occluded	1 (0.7)
	NA	1 (0.7)
Previous Ipsilateral Intervention (%)	NO	126 (88.1)
	YES	8 (5.6)
	NA	9 (6.3)
ASA Grade (%)	1	69 (48.3)
	2	28 (19.6)
	3	42 (29.4)
	4	3 (2.1)
	NA	1 (0.7)

NA, missing values; TIA, transitory ischemic attack; NYHA, New York Heart Association classification of heart failures. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

Table 4 | Clinical history, and anamnestic details from patients who underwent surgery for AAA.

Variable		**AAA patients (n = 56)**
Admission mode (%)	ELECTIVE	47 (83.9)
	NA	9 (16.1)
Sex (%)	Female	3 (5.4)
	Male	43 (76.8)
	NA	10 (17.9)
Hypertension History (%)	NO	6 (10.7)
	YES	40 (71.4)
	NA	10 (17.9)

Dyslipidemia (%)	NO	18 (32.1)
	YES	29 (51.8)
	NA	9 (16.1)
Diabetes (%)	NO	39 (69.6)
	YES	4 (7.1)
	NA	13 (23.2)
Cardiac History (%)	NO	22 (39.3)
	YES	24 (42.9)
	NA	10 (17.9)
Pulmonary History (%)	NO	34 (60.7)
	YES	12 (21.4)
	NA	10 (17.9)
Cerebrovascular disease (%)	NO	41 (73.2)
	YES	5 (8.9)
	NA	10 (17.9)
Creatine, mg/dL (median [IQR])		1.00 [0.90, 1.20]
Peripheral Arteriopathy (%)	NO	37 (66.1)
	YES	8 (14.3)
	NA	11 (19.6)
Smoke (%)	NO	22 (39.3)
	YES	23 (41.1)
	NA	11 (19.6)
Maximum Aortic Diameter, mm (median [IQR])		53.50 [45.00, 56.00]
AAA Type (%)	FUSIFORM	32 (57.1)
	SACCIFORM	10 (17.9)
	NA	14 (25.0)
Extension (%)	Aortic	23 (41.1)
	Aorto-bisiliac	8 (14.3)
	Aorto-iliac	10 (17.9)
	Iliac	1 (1.8)
	NA	14 (25.0)
Aortic Neck Diameter, mm (median [IQR])		23.00 [21.00, 24.55]
Aortic Neck Length, mm (mean (SD))		32.89 (15.41)
Right Iliac Artery Diameter, mm (median [IQR])		14.20 [12.00, 18.75]
Left Iliac Artery Diameter, mm (median [IQR])		14.05 [11.58, 19.38]

NA, missing values; SD standard deviation; IQR interquartile range. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

In the previous two tables, missing values were observed in various fields of the medical registry, and these gaps in data can occur due to reasons like incomplete patient records, data entry errors, administrative issues, data extraction problems, patient non-compliance, and lost or damaged records. To address this issue and ensure the completeness and accuracy of the data, specialized algorithms have been designed using the R software.

These algorithms facilitates quality control by automatically generating tables and figures that offer an overview of the data collected at any given moment during data collection. For instance, **Figure *1***, provides a visualization of the number of fields within each section of the database, accompanied by the respective percentage of missing values. This visual representation not only assists in pinpointing gaps in the data but also helps in prioritizing efforts to collect the missing information effectively.

Furthermore, the utilization of such algorithms is not limited to merely identifying missing values; they can also aid in tracking trends in data completeness over time, allowing for continuous improvement in data collection processes. By providing real-time insights into data quality, these tools enable healthcare professionals take corrective actions as needed.

The detailed data for other sections of the registry can be explored in the supplementary section, offering a comprehensive view of the entire dataset.

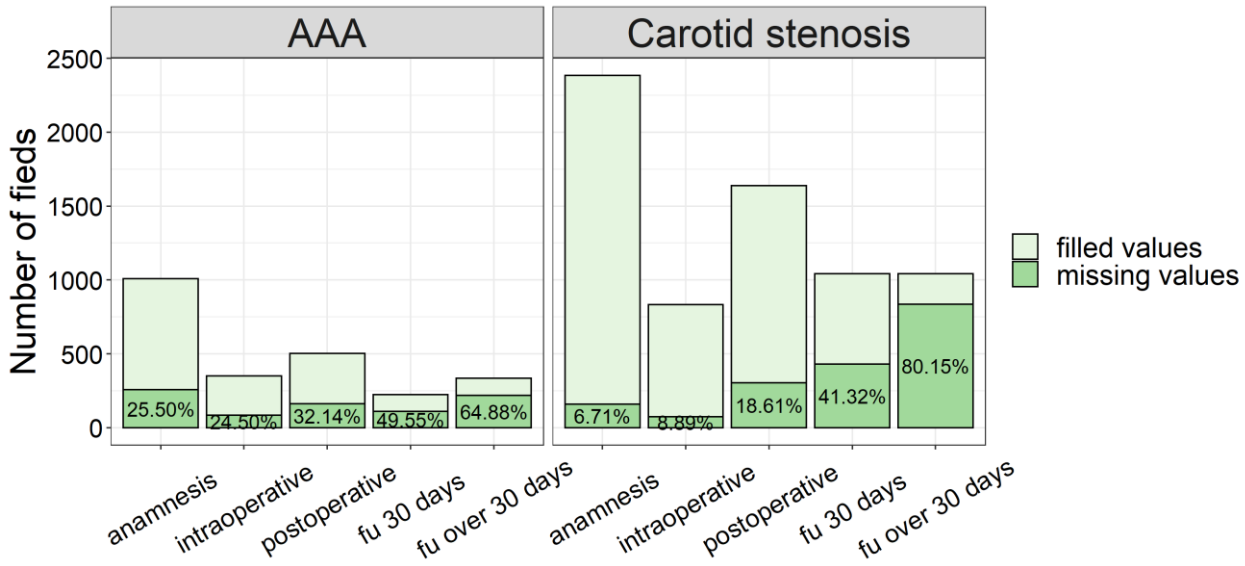

Figure 1| Bar chart illustration of the completeness of medical data records for AAA and Carotid Stenosis, across different section of the registry. Each bar is divided into two parts representing the proportion of filled values and missing values. Percentages indicate the proportion of missing data at each section.

Nrf2/AhR and HO-1 Pathways in AAA

In this section, we delve into exploration of the AhR, BMAL1, and the Nrf2/HO-1 pathways to shed light on the potential use of these biomolecules as biomarkers of AAA development.

Samples from GTB-VB cannot be utilized without prior institutional approval from San Martino Hospital and the institutional ethical committee. Protocol and necessary documentation for study evaluation were prepared and submitted, resulting in the successful acquisition of ethical and institutional approval.

Before obtaining this approval, preliminary experiments were conducted using samples obtained through a prior collaboration with the Leiden Vascular Biobank at Leiden University Medical Center in the Netherlands.

Real-time PCR measurements were employed to assess the expression levels of AhR and HO-1 in tissue samples harvested from the aortic walls of patients who presented varying stages of atherosclerotic lesions, alongside a comparison group consisting of patients afflicted with AAA. **Figure *2*** showcase that both AhR and HO-1 expression levels are diminished in AAA tissue samples in compared to atherosclerotic lesions.

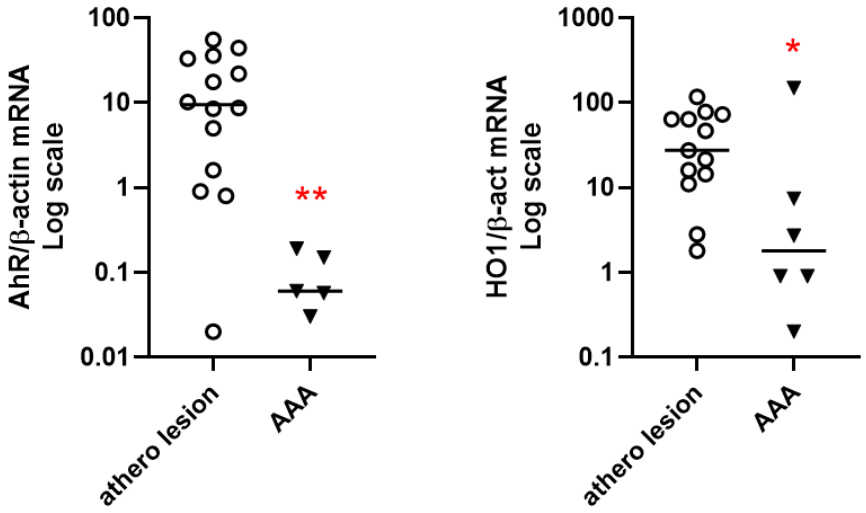

Figure 2 | Scatter plot of the expression levels measure with Real Time PCR of AhR and HO-1 mRNA relative to beta-actin mRNA in tissue samples from atherosclerotic lesions and AAA; * $p < 0.05$, ** $p < 0.01$.

To further investigate this finding, we conducted an analysis of AhR and HO-1 protein expression within histological sections from various lesions within the aortic wall.

In contrast to mRNA levels, we observed differential immunopositivity for AhR and HO-1 across lesions. These proteins exhibited a more pronounced presence in complex lesions, particularly in cases of AAA, as opposed to the relatively muted levels seen in the early atherosclerotic lesions such as adaptive intimal thickening, fibroatheroma, and intimal xanthoma (**Figure *3***).

Interestingly, the immunohistochemical analysis revealed a notable pattern wherein AhR and HO-1 tended to localize near sites undergoing remodeling within the aortic wall. These areas often coincided with small vessels undergoing neoangiogenesis and regions marked by infiltration of inflammatory cells.

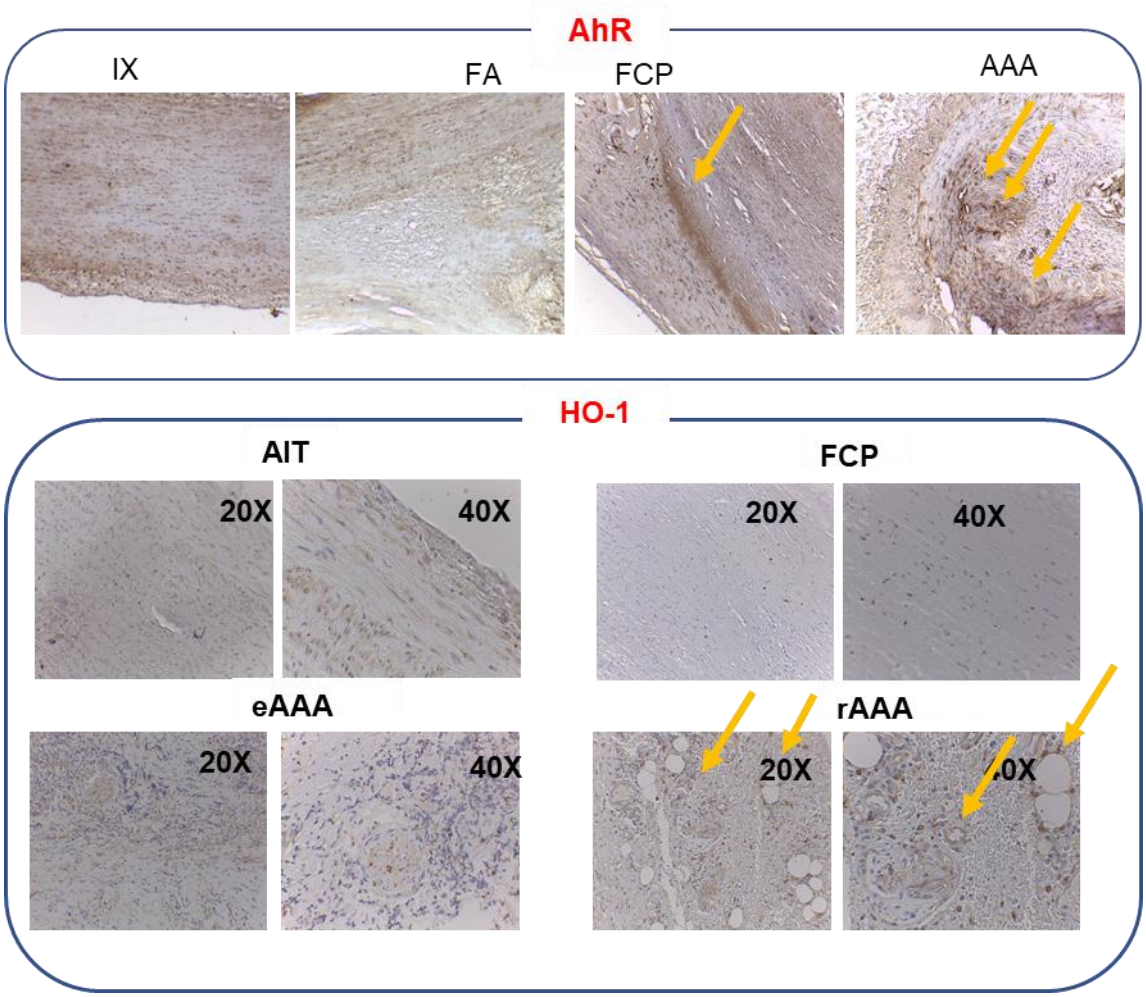

Figure 3 | Histopathology sections of human aortic wall belonging to patient in different stages of plaque evolution in AAA pathology. IX, intimal xanthoma; FA, fibroatheroma: FCP, fibrous calcific plaque; AIT, Adaptive intimal thickening; eAAA, elective abdominal aortic aneurysm; rAAA, rupture abdominal aortic aneurysm.

Following the approval of the study, we initiated the retrieval of plasma and PBMC samples from patients with AAA and carotid stenosis stored in the GTB-VB biobank. During this phase, we opted to use samples obtained from patients who had undergone surgery for carotid stenosis as our control group. This choice was driven by the similarity in age and risk factors between patients with carotid stenosis and those with AAA. **Table 5** provides an overview of the clinical characteristics of both study cohorts, highlighting the resemblance between the two patient populations. The only discernible difference between the two cohorts is the prevalence of diabetes, a known factor inversely associated with AAA[22].

Table 5 | Patient demographics and comorbidities of the study population.

Variable	AAA patients (n = 44)	Carotid stenosis patients (n = 62)	*P* value
Age (median [IQR])	74,50 [69,75, 80,25]	76,00 [72,00, 80,75]	0,517
Diabetes (%)	5 (11,4)	17 (27,4)	0,045
Cardiac History (%)	9 (20,5)	6 (9,7)	0,117
Dyslipidemia (%)	20 (45,5)	26 (41,9)	0,719
Hypertension History (%)	24 (54,5)	32 (51,6)	0,766
Chronic Kidney Insufficiency (%)	5 (11,4)	6 (9,7)	0,779
Peripheral artery disease (%)	1 (2,3)	5 (8,1)	0,204
Leukocytes x10E9/L (mean (SD))	7,23 (2,02)	6,99 (1,67)	0,538
Erythrocytes x10E12/L (mean (SD))	4,70 [4,30, 4,90]	4,40 [3,95, 4,70]	0,024
Hemoglobin g/L (mean (SD))	136,07 (17,34)	129,34 (17,53)	0,076
Platelets x10E9/L (mean (SD))	199,00 [178,00, 227,75]	192,00 [167,00, 235,50]	0,621
Neutrophils x10E9/L (median [IQR])	4,57 [4,07, 5,50]	4,77 [3,96, 5,31]	0,831
Lymphocytes x10E9/L (median [IQR])	1,41 [1,07, 1,69]	1,30 [1,00, 2,14]	0,736
Creatinine mg/dL (median [IQR])	1,00 [0,90, 1,30]	1,10 [0,90, 1,30]	0,544
eGFR mL/m^2/1,73mq (median [IQR])	69,00 [54,50, 82,00]	66,00 [52,00, 82,00]	0,542
INR (median [IQR])	1,06 [1,00, 1,21]	1,03 [0,97, 1,10]	0,152

eGFR estimated glomerular filtration rate; INR international normalized ratio; SD standard deviation; IQR interquartile range. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing. Comparison of Gaussian distributed data was done using unpaired t test, non-Gaussian distributed by Wilcoxon Rank Sum Test. Comparison of prevalence for risk factors was analyzed by χ^2 test.

Enzyme-linked immunosorbent assay (ELISA) tests were perform of samples of 106 subjects. Specifically, the ELISA assay was carried out serum samples from 44 patients with AAA and 62 samples from patients with carotid stenosis.

Figure 4 depicts a higher median level of serum HO-1 in patients with Carotid Stenosis compared to those with AAA. The difference between the two groups is statistical significance with a p-value of 0.0083.

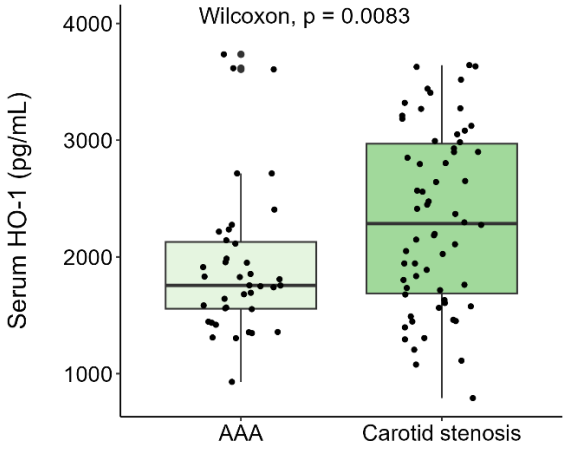

Figure 4 | Boxplot and scatter plot displaying serum HO-1 levels expression in AAA and carotid stenosis patients. The plot reveals a statistically significant higher median concentration of HO-1 in the Carotid Stenosis group compared to the AAA group, with a p-value of 0.0083. Wilcoxon signed-ranked test was used to compare the two samples population.

In PBMCs, mRNA expression measurements were conducted for three target genes of the Nrf2/AhR pathway, namely HO1, AhR, and Bmal. Contrary to what was observe in the protein expression the levels of relative mRNA expression of these target genes between the two cohorts did not reveal any statistically significant differences the two populations (**Figure *5***). The p-values are well above the conventional threshold of 0.05, suggesting that the mRNA levels of these genes are similar across the two patient groups. This lack of significant difference at the mRNA level is noteworthy given the previous observation of differential serum protein levels. It raises the possibility of post-transcriptional or post-translational modifications or varying cellular activation states that might influence serum protein levels independently of mRNA expression. Alternatively, the decrease in HO-1 serum levels in AAA patients may not be attributed to PBMCs as the source; it could reflect its consumption at the site of the lesion, suggesting localized damage with potential systemic consequences.

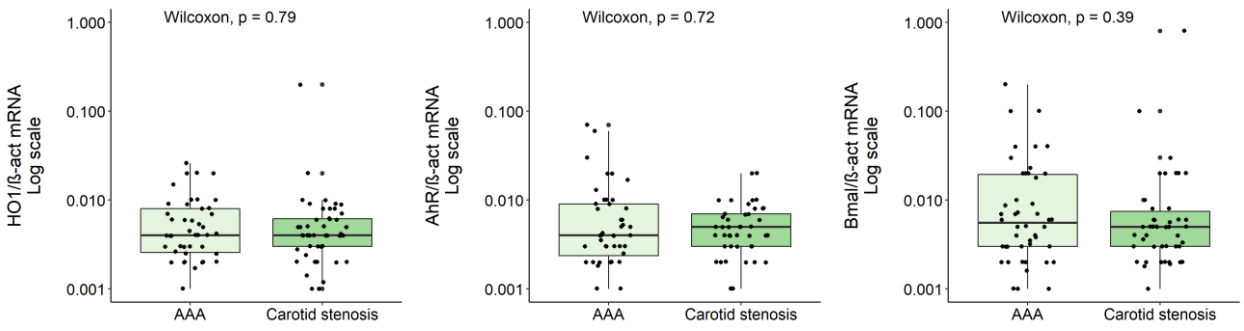

Figure 5 | Set of box and scatter plots comparing the relative expression levels of HO-1, AHR, and Bmal1 mRNA to beta-actin mRNA between patients with AAA and Carotid Stenosis. Expression levels are presented on a logarithmic scale, with each plot showing no significant difference between the two conditions, as indicated by the p-values, 0.79, 0.72, and 0.39 respectively. Wilcoxon signed-ranked test was used to compare the two samples population.

Although previous observations indicated that the two cohorts share common risk factors and exhibit similarities in basic blood laboratory results, it's important to acknowledge that they do differ

statistically in terms of diabetes prevalence and median erythrocyte counts. To address the possibility that variations in HO-1 expression levels could be attributed to these differences, we conducted regression analyses involving serum HO-1 levels, erythrocyte count, age, and creatinine levels.

The results of the regression analyses reveal only a weak correlation between serum HO-1 levels and these three measured parameters, as indicated by the relatively low R-squared values ranging from 0.031 to 0.037 (**Figure *6***). This suggests that while there might be a subtle trend of increasing HO-1 levels with advancing age, renal function, and erythrocyte count, these factors alone do not serve as robust predictors of HO-1 levels. Consequently, this implies that the role of HO-1 and its regulatory mechanisms in these medical conditions are not significantly influenced by these variables.

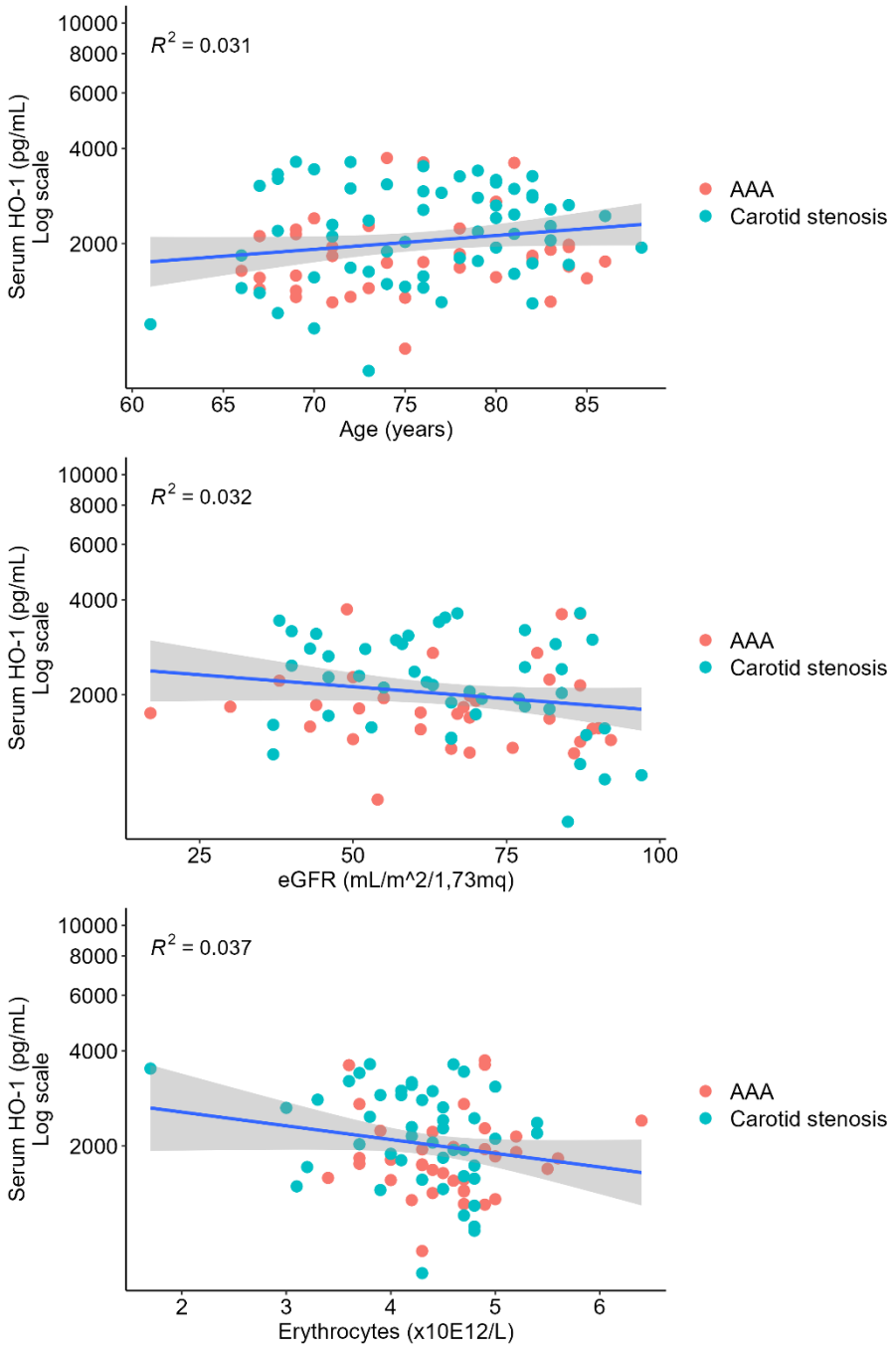

Figure 6 | Set of scatter plots illustrating the correlation between serum HO-1 levels and age, eGFR (estimated Glomerular Filtration Rate), and erythrocyte count in patients with Abdominal Aortic Aneurysm (AAA) and Carotid Stenosis. Each plot shows a regression line with a shaded confidence interval, indicating a weak correlation with R^2 values of 0.031, 0.032, and 0.037, respectively.

The relatively lower frequency of diabetes within the AAA cohort, led us to perform a separate analysis by dividing the study population into subgroups based on the presence or absence of diabetes. Subsequent comparison of serum HO-1 levels between these subgroups revealed no statistically significant differences, as indicated by p-values exceeding 0.9 when employing the Wilcoxon rank sum test. This finding suggests that the presence or absence of diabetes does not appear to be a significant factor contributing to variations in serum HO-1.

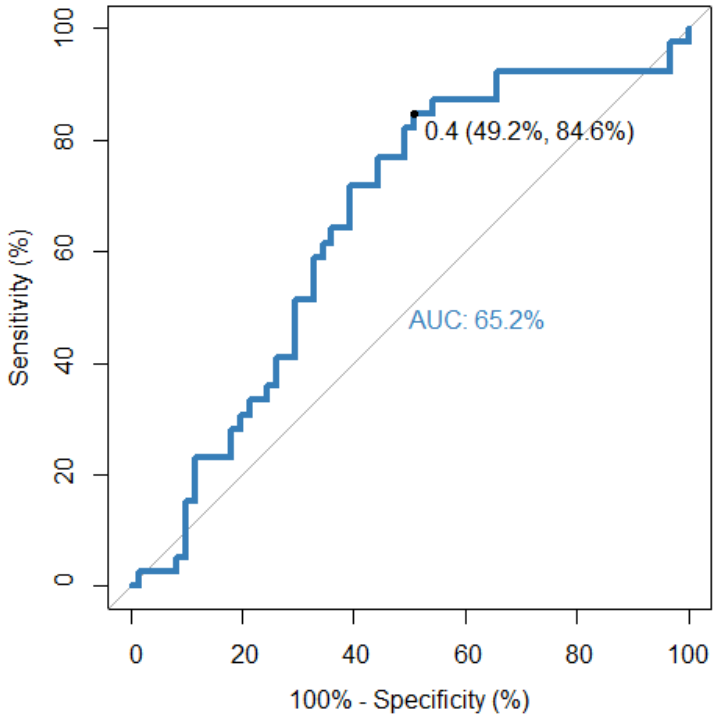

Figure 7 | ROC curve analysis of a regression model predicting the presence of Abdominal Aortic Aneurysm (AAA) versus Carotid Stenosis using serum HO-1 levels as the predictor. The AUC of 65.2% suggests a modest predictive ability, with an identified optimal threshold (indicated by the square marker) corresponding to 49.2% specificity and 84.6% sensitivity.

The ROC curve in **Figure 7** showcases the capability of serum HO-1 levels to discriminate between the patients with AAA and patients with Carotid Stenosis. With an Area Under the Curve (AUC) of 65.2%, the model demonstrates a modest predictive power. This level of AUC indicates that while serum HO-1 can be used to differentiate between AAA and Carotid Stenosis to some degree, its accuracy is somewhat limited and may not be reliable as a standalone diagnostic tool. The optimal threshold identified on the curve achieves an 84.6% sensitivity rate, which is relatively high, indicating that the model is adept at identifying the true positives for AAA. However, this comes at the cost of specificity, which is only 49.2%, signifying that almost half of the Carotid Stenosis patients would be incorrectly classified as having AAA at this threshold.

These insights suggest that while serum HO-1 levels do carry some predictive value for AAA, they also present substantial limitations. The moderate AUC and the trade-off between sensitivity and specificity imply that HO-1 should be part of a more comprehensive panel of biomarkers and clinical features to effectively identify AAA. The reliance on a single biomarker like HO-1 could lead to a significant number of false positives or false negatives, which is not ideal for clinical decision-making. Therefore, incorporating additional predictors and perhaps utilizing advanced modeling techniques could improve diagnostic accuracy and the clinical utility of predicting AAA in patients with vascular abnormalities.

Discussion

Insights into AAA Pathophysiology and Biomarker Exploration

Our study, delving into the Nrf2/AhR and HO-1 pathways, cautiously hints at their potential involvement in the pathophysiology of abdominal aortic aneurysms. The differential expression of AhR and HO-1 in tissue and serum samples, coupled with their distinct histopathological localizations, suggests a possible role in AAA development.

In the analysis of PBMCs, the expression of Nrf2/AhR pathway genes (HO1, AhR, and Bmal) did not exhibit statistically significant differences in mRNA levels between AAA and carotid stenosis cohorts. The lack of disparity at the mRNA level, despite variations in serum protein levels, may suggests regulatory mechanisms beyond transcription. This may involve post-transcriptional or post-translational modifications or diverse cellular activation states affecting protein levels independently of mRNA expression. Furthermore, the observed decrease in HO-1 serum levels in AAA patients, not mirrored in PBMC mRNA levels, opens the possibility that these changes might be attributed to consumption at the lesion site.

The study's findings regarding serum HO-1 levels in differentiating AAA from carotid stenosis, offer an intriguing avenue for further exploration. Although the ROC analysis suggests a modest predictive ability of serum HO-1, it also underscores the limitations of relying on a single biomarker in isolation. In the context of personalized medicine, these findings reinforce the need for a holistic, multi-parametric approach to disease diagnosis and risk stratification, integrating various clinical and molecular markers.

The Role and Evolution of Biobanking in Vascular Research

The establishment and diligent management of the GTB-VD biobank represent biobank mark a progressive step in vascular disease research. This manuscript illustrates the potential unlocked through the systematic collection of a diverse array of biospecimens, coupled with the implementation of robust data management practices. In this study, the biobank has been instrumental in facilitating the exploration of biological markers associated with AAA. This rich repository of biological data has allowed our study to probe into the molecular aspects of AAA within a clinical framework, providing valuable insights that may guide future research.

The GTB-VD, since its inception in 2018, represents a model of modern biobanking, contributing to collaborative research in complex diseases such as atherosclerosis. Its partnership with various research units within the “Rete Cardiologica” network exemplifies the efforts to maintain consistency and adherence to shared procedures and ethical guidelines. Such collaborative endeavors are instrumental in unearthing novel diagnostic and therapeutic targets, especially in diseases lacking specific biomarkers.

Future Perspectives and Limitations

While our study provides valuable insights, it is not devoid of limitations. The presence of missing data and potential confounding factors, such as the higher diabetes prevalence in the carotid stenosis group, calls for a careful interpretation of results. Future research should aim to mitigate these limitations by incorporating

larger and more diverse patient cohorts and employing advanced analytical techniques. This approach will ensure a more robust understanding of the complex mechanisms underlying AAA.

In conclusion, our study cautiously proposes the involvement of the Nrf2/AhR and HO-1 pathways in AAA's pathogenesis and highlights the prospective role of serum HO-1 as a biomarker in vascular diseases. The findings also accentuate the critical role of biobanks in propelling vascular disease research. As we move forward, a multidisciplinary strategy integrating molecular biology, clinical data, and sophisticated analytics will be pivotal in unraveling AAA's complex mechanisms and enhancing patient care. The ongoing evolution and integration of biobanks into biomedical research remain essential in addressing multifaceted diseases' complexities and ultimately advancing patient care.

Bibliography

1. Quintana, R. A. & Taylor, W. R. Cellular Mechanisms of Aortic Aneurysm Formation. *Circ. Res.* **124**, 607–618 (2019).
2. Antoniou, G. A., Antoniou, S. A. & Torella, F. Editor's Choice - Endovascular vs. Open Repair for Abdominal Aortic Aneurysm: Systematic Review and Meta-analysis of Updated Peri-operative and Long Term Data of Randomised Controlled Trials. *Eur. J. Vasc. Endovasc. Surg. Off. J. Eur. Soc. Vasc. Surg.* **59**, 385–397 (2020).
3. Lederle, F. A. *et al.* Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. *Ann. Intern. Med.* **126**, 441–449 (1997).
4. Davis, F. M., Rateri, D. L. & Daugherty, A. Abdominal aortic aneurysm: novel mechanisms and therapies. *Curr. Opin. Cardiol.* **30**, 566–573 (2015).
5. Wu, X. *et al.* Sex- and disease-specific inflammasome signatures in circulating blood leukocytes of patients with abdominal aortic aneurysm. *Mol. Med. Camb. Mass* **22**, 505–518 (2016).
6. Chen, Z. *et al.* Inhibition of Nrf2/HO-1 signaling leads to increased activation of the NLRP3 inflammasome in osteoarthritis. *Arthritis Res. Ther.* **21**, 300 (2019).
7. Hennig, P. *et al.* The Crosstalk between Nrf2 and Inflammasomes. *Int. J. Mol. Sci.* **19**, 562 (2018).
8. Huai, W. *et al.* Aryl hydrocarbon receptor negatively regulates NLRP3 inflammasome activity by inhibiting NLRP3 transcription. *Nat. Commun.* **5**, 4738 (2014).
9. Eckers, A. *et al.* The aryl hydrocarbon receptor promotes aging phenotypes across species. *Sci. Rep.* **6**, 19618 (2016).
10. Enoki, Y. *et al.* Indoxyl sulfate potentiates skeletal muscle atrophy by inducing the oxidative stress-mediated expression of myostatin and atrogin-1. *Sci. Rep.* **6**, 32084 (2016).
11. Ma, Q. Role of Nrf2 in Oxidative Stress and Toxicity. *Annu. Rev. Pharmacol. Toxicol.* **53**, 401–426 (2013).

12. Kopacz, A. *et al.* Simvastatin Attenuates Abdominal Aortic Aneurysm Formation Favoured by Lack of Nrf2 Transcriptional Activity. *Oxid. Med. Cell. Longev.* **2020**, 6340190 (2020).
13. Nitti, M., Furfaro, A. L. & Mann, G. E. Heme Oxygenase Dependent Bilirubin Generation in Vascular Cells: A Role in Preventing Endothelial Dysfunction in Local Tissue Microenvironment? *Front. Physiol.* **11**, 23 (2020).
14. Azuma, J. *et al.* Heme Oxygenase-1 Expression Affects Murine Abdominal Aortic Aneurysm Progression. *PLOS ONE* **11**, e0149288 (2016).
15. Ho, Y.-C. *et al.* Heme oxygenase-1 deficiency exacerbates angiotensin II-induced aortic aneurysm in mice. *Oncotarget* **7**, 67760–67776 (2016).
16. Schillinger, M. *et al.* Heme oxygenase-1 gene promoter polymorphism is associated with abdominal aortic aneurysm. *Thromb. Res.* **106**, 131–136 (2002).
17. Hofmann, A. *et al.* Pharmacotherapies and Aortic Heme Oxygenase-1 Expression in Patients with Abdominal Aortic Aneurysm. *Antioxid. Basel Switz.* **11**, 1753 (2022).
18. Kishimoto, Y., Kondo, K. & Momiyama, Y. The Protective Role of Heme Oxygenase-1 in Atherosclerotic Diseases. *Int. J. Mol. Sci.* **20**, 3628 (2019).
19. Chng, S. H. *et al.* Ablating the aryl hydrocarbon receptor (AhR) in CD11c+ cells perturbs intestinal epithelium development and intestinal immunity. *Sci. Rep.* **6**, 23820 (2016).
20. Chhunchha, B., Kubo, E. & Singh, D. P. Clock Protein Bmal1 and Nrf2 Cooperatively Control Aging or Oxidative Response and Redox Homeostasis by Regulating Rhythmic Expression of Prdx6. *Cells* **9**, 1861 (2020).
21. Montecucco, F. *et al.* Implementation strategies of Systems Medicine in clinical research and home care for cardiovascular disease patients. *Eur. J. Intern. Med.* **25**, 785–794 (2014).
22. Radak, D., Tanaskovic, S., Katsiki, N. & Isenovic, E. R. Protective Role of Diabetes Mellitus on Abdominal Aortic Aneurysm Pathogenesis: Myth or Reality? *Curr. Vasc. Pharmacol.* **14**, 196–200 (2016).

Supplementary tables

Supplementary Table 1 | Intraoperative details of patients that underwent AAA surgical treatment.

Variable		Overall (n = 56)
Duration Operation, min (median [IQR])		100.00 [76.25, 190.00]
Anaesthetic (%)	General	30 (53.6)
	Local/Regional	12 (21.4)
	NA	14 (25.0)
Type Of Surgery (%)	ENDO	37 (66.1)
	OPEN	7 (12.5)
	NA	12 (21.4)

NA, missing values; SD standard deviation; ENDO, endovascular surgical treatment; OPEN, open surgical treatment. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

Supplementary Table 2 || Intraoperative details of patients that underwent AAA endovascular surgical treatment.

Variable		ENDO (n = 37)
Contrast Agent, mL (median [IQR])		60.00 [0.00, 133.50]
Access Type (%)	Hybrid	2 (3.6)
	Percutaneous	33 (58.9)
	Surgical exposure	2 (3.6)
	NA	19 (33.9)
Adjunctive Procedures (%)	NO	6 (10.7)
	SI	31 (55.4)
	NA	19 (33.9)
Conversion (%)	NO	37 (66.1)
	NA	19 (33.9)

NA, missing values; SD standard deviation. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

Supplementary Table 3 || Intraoperative details of patients that underwent AAA open surgical treatment.

Variable		OPEN (n = 7)
Suprarenal Clamp (%)	NO	5 (8.9)
	SI	2 (3.6)
	NA	49 (87.5)
Type of Intervention (%)	Aorto-aortic	4 (7.1)
	Aorto-bisiliac	3 (5.4)
	NA	49 (87.5)
Adjunctive Procedures (%)	NO	5 (8.9)
	SI	1 (1.8)
	NA	50 (89.3)

NA, missing values.

Supplementary Table 4 | Post-operative details of patients that underwent AAA surgical treatment.

Variable		Overall (n = 56)
Acute Coronary Event (%)	NO	43 (76.8)
	NA	13 (23.2)
Haemorrhage (%)	NO	42 (75.0)
	NA	14 (25.0)
Postoperative Renal Failure (%)	NO	41 (73.2)
	TEMPORARY	2 (3.6)
	NA	13 (23.2)
Renal Replacement Therapy (%)	NO	42 (75.0)
	NA	14 (25.0)
Respiratory Failure (%)	NO	42 (75.0)
	SI	1 (1.8)
	NA	13 (23.2)
Bowel Ischemia (%)	NO	42 (75.0)
	NA	14 (25.0)
Intensive Care (%)	NO	1 (1.8)
	NA	55 (98.2)
Discharge Status (%)	ALIVE	43 (76.8)
	NA	13 (23.2)
Return To Theatre Prior Discharge (%)	NO	43 (76.8)
	NA	13 (23.2)

NA, missing values.

Supplementary Table 5 |Follow up details of patients that underwent surgical treatment for AAA after 30 days of the procedures.

Variable		Overall (n = 56)
Status 30 days (%)	ALIVE	29 (51.8)
	NA	27 (48.2)
Procedure Related Systemic Complications 30 days (%)	NO	28 (50.0)
	NA	28 (50.0)
Related Complications 30 days (%)	NO	28 (50.0)
	NA	28 (50.0)
Reintervention 30 days (%)	NO	28 (50.0)
	NA	28 (50.0)

NA, missing values.

Supplementary Table 6 | Follow up details of patients that underwent surgical treatment for AAA over 30 days of the procedure.

Variable		Overall (n = 56)
Status Over 30 days (%)	ALIVE	15 (26.8)
	NA	41 (73.2)
Months Post Discharge Over 30 days (median [IQR])		0.00 [0.00, 0.00]
Procedure Related Systemic Complications Over 30 days (%)	NO	14 (25.0)
	NA	42 (75.0)
Graft Related Complications Over 30 days (%)	NO	15 (26.8)
	NA	41 (73.2)
Graft Infection (%)	NO	15 (26.8)
	NA	41 (73.2)
Reintervention Over 30 days (%)	NO	14 (25.0)
	SI	1 (1.8)
	NA	41 (73.2)

NA, missing values; SD standard deviation. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

Supplementary Table 7 | Intraoperative details of patients that underwent surgical treatment for carotid stenosis.

Variable		Overall (n = 143)
Duration Operation, min (median [IQR])		95.00 [60.00, 130.00]
Procedure (%)	BYPASS	2 (1.3)
	CEA	107 (71.8)
	STENT	32 (21.5)
	NA	8 (5.4)
Anaesthetic (%)	General	109 (73.2)
	Local/Regional	28 (18.8)
	NA	12 (8.1)

NA, missing values; SD standard deviation; CEA, carotid endarterectomy; CAS, carotid stenting. All data are presented as the median [IQR] or as mean (SD) depending on the result of normality testing.

Supplementary Table 8 | Intraoperative details of patients that underwent carotid endarterectomy.

Variable		CEA (n = 107)
CEA type (%)	Conventional CEA	1 (0.7)
	Eversion CEA	103 (69.1)
	NA	45 (30.2)
Patch (%)	NO	83 (55.7)
	SI	6 (4.0)
	NA	60 (40.3)
Shunt (%)	JAVID	1 (0.7)
	NO	79 (53.0)
	PRUITT	3 (2.0)
	NA	66 (44.3)

NA, missing values; CEA, carotid endarterectomy.

Supplementary Table 9 | Intraoperative details of patients that underwent carotid stenting.

Variable		CAS (n = 32)
Stent Type (%)	Closed Cells	1 (0.7)
	Micromesh	18 (12.1)
	Open Cells	13 (8.7)
	NA	117 (78.5)
PTA Stent (%)	Filter	29 (19.5)
	Proximal Occlusion	1 (0.7)
	Without protection	1 (0.7)
	NA	118 (79.2)

NA, missing values; CAS, carotid stenting; PTA, percutaneous transluminal angioplasty.

Supplementary Table 10 | Post-operative details of patients that underwent surgical treatment for carotid stenosis.

Variable		Overall (n = 143)
Haemorrhage (%)	NO	114 (76.5)
	YES - NO REINTERVENTION	4 (2.7)
	YES - REINTERVENTION	3 (2.0)
	NA	28 (18.8)
Angiography (%)	NO	107 (71.8)
	SI	21 (14.1)
	NA	21 (14.1)
Color Ultrasound (%)	NO	9 (6.0)
	SI	118 (79.2)
	NA	22 (14.8)
Post Grade Stenosis (%)	0 - 49 %	79 (53.0)
	50 - 69 %	1 (0.7)
	70 - 99 %	5 (3.4)
	NA	64 (43.0)
Post Grade Contralateral Stenosis (%)	0 - 49 %	67 (45.0)
	50 - 69 %	26 (17.4)
	70 - 99 %	4 (2.7)
	Occluded	1 (0.7)
	NA	51 (34.2)
TIA (%)	NO	131 (87.9)
	SI	2 (1.3)
	NA	16 (10.7)
Stroke (%)	DISABLING	1 (0.7)
	NO	115 (77.2)
	NA	33 (22.1)
Cerebral Haemorrhage (%)	NO	134 (89.9)
	NA	15 (10.1)
	NO	113 (75.8)
	NA	32 (21.5)
Any Major Cardiac Event (%)	CARDIAC FAILURE	2 (1.3)
	SERIOUS ARRHYTHMIA	2 (1.3)
Nerve (%)	NO	122 (81.9)
	SI	15 (10.1)
	NA	12 (8.1)
Discharge Status (%)	ALIVE	138 (92.6)
	NA	11 (7.4)

NA, missing values; TIA, transitory ischemic attack.

Supplementary Table 11 | Follow up details of patients that underwent surgical treatment for carotid stenosis after 30 days of the procedure.

Variable		Overall (n = 143)
Status 30 days (%)	ALIVE	71 (47.7)
	NA	78 (52.3)
Haemorrhage 30 days (%)	NO	140 (94.0)
	YES - NO REINTERVENTION	1 (0.7)
	NA	8 (5.4)
TIA 30 days (%)	NO	71 (47.7)
	NA	78 (52.3)
Stroke 30 days (%)	NO	71 (47.7)
	NA	78 (52.3)
Cerebral Haemorrhage 30 days (%)	NO	71 (47.7)
	NA	78 (52.3)
Any Major Cardiac Event 30 days (%)	NO	113 (75.8)
	NA	32 (21.5)
	CARDIAC FAILURE	2 (1.3)
	SERIOUS ARRHYTHMIA	2 (1.3)
Nerve 30 days (%)	NO	67 (45.0)
	SI	3 (2.0)
	NA	79 (53.0)

NA, missing values; TIA, transitory ischemic attack.

Supplementary Table 12 | Follow up details of patients that underwent surgical treatment for carotid stenosis over 30 days of the procedure.

Variable		Overall (n = 143)
Status Over 30 days (%)	ALIVE	11 (7.4)
	NA	138 (92.6)
Haemorrhage Over 30 days (%)	NO	141 (94.6)
	NA	8 (5.4)
TIA Over 30 days (%)	NO	11 (7.4)
	NA	138 (92.6)
Stroke Over 30 days (%)	NO	11 (7.4)
	NA	138 (92.6)
Cerebral Haemorrhage Over 30 days (%)	NO	11 (7.4)
	NA	138 (92.6)
Any Major Cardiac Event Over 30 days (%)	NO	11 (7.4)
	NA	138 (92.6)
Nerve Over 30 days (%)	NO	11 (7.4)
	NA	138 (92.6)

NA, missing values; TIA, transitory ischemic attack.