The Genoa Vascular Biobank: A today resource for future perspectives in vascular research

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Abstract: The Genoa Vascular Biobank (GTB - VD) is a collaborative network among the IRCCS Ospedale Policlinico San Martino (Centre of Biological Resources), and the University of Genoa (Vascular and Endovascular Surgery and of Anatomic Pathology Units and the Laboratory of Clinical and Experimental Vascular Biology).

Patients undergoing elective surgery for carotid artery stenosis (CS) and abdominal aortic aneurysm (AAA) are enrolled on the basis of inclusion and exclusion criteria, after a signed informed consent, and subdivided for pathology and surgical approach (open or endovascular).

Biospecimens comprise serum, plasma, whole blood, peripheral blood mononuclear cells and urine (from AAA only), stored at -80°C; lesions from open surgeries are frozen and formalin fixed-paraffin embedded.

Samples are associated with donor's clinical data through pseudonymization to prevent patient identification. Data accuracy and sample quality are ensured by harmonized standard operative procedures.

To date, GTB - VD comprises 351 CS and 186 AAA. CS patients are further distinguished into symptomatic or asymptomatic, displaying a ratio of 5:1.

The present work describes the whole workflow, the ethic and governance requirements, the demographic and clinical characteristics of subjects enrolled in the biobank and the volume of open or endovascular surgical techniques.

GTB - VD, as organized, represents a unicum in our Country. It supports studies to identify molecular targets and biomarkers associated with specific arteriopathy, for the development of secondary prevention strategies and minimally invasive, in situ therapies. Collaborative studies and sample sharing are welcome.

Keywords: Vascular biobank; Vascular Surgery; Abdominal Aortic Aneurysm; Atherosclerosis; Vulnerable Plaque; Carotid Endoarterectomy - CEA; Carotid Artery Stenting - CAS; Endovascular Aortic Repair - EVAR; Open Surgical Repair - OSR; Symptomatic carotid stenosis; Severe carotid stenosis; ischemic stroke; peripheral blood-derived samples

1. INTRODUCTION

Over the past two decades, advancements in biological knowledge and patient care have been significantly enhanced by the emergence of -omics sciences, including genomics, transcriptomics, proteomics, and metabolomics, along with the field of Systems Medicine.

Systems Medicine is defined as 'an integrative approach to medical needs that leverages and emphasizes information and tools from a wide spectrum of scientific disciplines. Its primary aim is to enhance risk prediction and individualized treatment while respecting ethical and legal requirements'. These innovative disciplines demand a high degree of quality, traceability of biospecimens, and standardization of data collection to ensure the generation of comparable data and the integration of information for analysis.

To meet these demands, biobanks have evolved from simple biological sample repositories into complex and dynamic units integrated into multi-organizational infrastructure networks. Academic Medical Centers are ideally positioned to establish and manage biobanks due to their ability to provide access to human subjects, biological specimens, and clinical data. Furthermore, biobanking activities align with their mission of education, research, and patient care [1].

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

Our research unit (Vascular and Endovascular Surgery unit) embarked on this journey approximately 15 years ago by collecting biological specimens and clinical-demographic data from diverse patient cohorts suffering from vascular diseases such as varicose veins and artery diseases [2-15]. This ongoing activity has fueled collaborative research projects with institutional, national, and international teams.

In particular, we have collected biospecimens and data from candidates undergoing elective surgery for carotid artery stenosis (CS) and abdominal aortic aneurysm (AAA). The high number of these interventions is motivated by the need to prevent the potentially devastating consequences of ischemic

stroke, due to carotid plaque vulnerability and abdominal aortic rupture, on the basis of a statistical risk stratification calculated from morphometrical parameters on arterial imaging.

The paucity of information related to the biological mechanisms responsible of such major adverse events, of specific biomarkers for risk stratification and of therapeutic targets, prompted us to establish, in 2018, the Genoa Vascular Biobank, which enroll CS and AAA patients. Systematically, subjects are selected at the recruitment on the basis of inclusion and exclusion criteria, biological specimens are collected under controlled conditions and personal data are organized as reported in this work.

In this manuscript, our goal is to provide an overview of the network, workflow, ethical considerations, and legal aspects of the Genoa Tissue Bank – Vascular Division (GTB-VD), along with a summary of the resources accumulated to date. The primary mission of the GTB-VD is to contribute to the identification of molecular targets and circulating biomarkers specifically associated with atherosclerotic complications. Additionally, it aims to support future developments in the field of artery diseases, providing more accurate parameters for risk stratification and personalized interventions.

2. MATERIALS & METHODS

2.1 Network composition and Workflow

The GTB-VD initiative encompasses several key components, including the Vascular and Endovascular Surgery Unit, the Laboratory of Experimental and Clinical Vascular Biology (known as BioVasc Lab), and the Anatomic Pathology Unit within the Department of Surgical and Diagnostic Sciences at the University of Genoa. Additionally, storage facilities are managed and coordinated by the Biological Resource Center (CRB-HSM) at the IRCCS Ospedale Policlinico San Martino.

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

The 3-step workflow proceeds as follow:

1. Selection and Recruitment of Participants and Biospecimen Collection:

Patients admitted to elective surgery for carotid stenosis or AAA, if eligible for biobank, are selected at the admission to the Vascular and Endovascular Surgery Unit one day before the intervention. They are offered the opportunity to participate in the Biobank and subsequently undergone the appropriate informed consent process. Blood and urine samples from participants are collected one day prior to 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 sample quality, including 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 off 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 described below in “Biospecimen collection and processing” paragraph.

3. Storage:

Frozen tissue samples are securely stored in monitored and alarmed facilities at -80°C, managed by the 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 housed within the prospective electronic database system of the Italian Society of Vascular and Endovascular Surgery (https://www.sicvereg.it/).

2.2 Ethics and governance of Genoa Tissue Bank -VD

The Vascular Bank Division (VD) is an integral part of the Clinical Pathology Tissue Bank (Genoa Tissue Bank -GTB) of the IRCCS Policlinico San Martino Hospital and belongs to the Institute's Biological Resources Center (CRB-HSM), together with the Neurological Bank (BioNeuro) and the Cell Bank (ICLC).

Samples and data collected and stored in the CRB are accessible to the scientific community through a clear access procedure compliant with the indications of the GDPR (REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27th April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation - GDPR) and the general access conditions of the BBMRI ERIC (Home - BBMRI-ERIC: Making New Treatments Possible), the consortium of biobanks of which the CRB is part.

Access to samples and data also respects the commitments made to donors and follows the principles of "equitable access" and scientific excellence. Access in the context of research projects is in fact permitted only for specific research projects, according to the terms of the consent given by the participant. All projects are subject to approval by the institutional Research Ethics Committee (REC) and by the internal CRB-HSM Evaluation Committee.

The collection of biological samples for research purposes occurs only after obtaining a written informed consent from the Participant, who has previously been provided with adequate information (also pursuant to the Privacy Code and the Provisions of the Privacy Guarantor). Information leaflets and consent forms are published by the Institution (CONSAZHQA_0015_GB-Biobanche_Centro_Risorse_Biologiche_(CRB)_Informativa_ai_sensi_degli_artt._12_13_14_Regolamento_UE_2016_679_INGLESE.PDF). Consent is collected before blood withdrawal and surgery. The documented informed consent is included as an integral part of the patient's medical record. A copy of the consent in paper and/or electronic format is adequately archived by CRB, kept in a place accessible only to duly appointed personnel and processed in compliance with current legislation on personal data.

Consent (authentic, free, autonomous, convinced, responsible) must always be revocable without any consequences for the Participant. In case of revocation, all data entered in the CRB-HSM will be deleted and the biological sample will be destroyed, anonymized or made available to the Participant for collection.

To comply with the GDPR, the Italian state has required that informed consent for the collection and distribution of samples takes place in reference to a specific research project; if the sample is requested for projects with purposes other than that of collection, the Participant will need to be contacted again to obtain a specific authorization to distribution and use. To overcome this difficult process, we are working to produce a "dynamic consent" procedure, as proposed in 2008 in the Ensuring Consent and Revocation project (EnCoRe - Ensuring Consent and Revocation (hp.com)), in order to make easier for people to turn consent decisions on and off.

2.3 Pre-surgery evaluation

Prior to surgery, all patients undergo a comprehensive preoperative evaluation conducted by a multidisciplinary team. This evaluation includes a range of assessments such as blood tests, electrocardiograms, chest x-rays, cardiology consultations, and risk stratification. Depending on the patient's comorbidities, additional examinations may be performed. For instance, patients with renal failure may undergo nephrological examinations, while those with chronic obstructive pulmonary disease may have pneumological assessments and pulmonary function tests. Patients scheduled for open AAA treatment also undergo preoperative respiratory function tests to optimize their respiratory health and minimize the risk of postoperative complications. Patients at high cardiac risk may undergo advanced investigations like echocardiography, coronary CT scans, and coronary angiography to address any underlying cardiac conditions.

– *Carotid Artery Stenosis*: patients diagnosed with CS receive a duplex ultrasound evaluation to assess the degree of stenosis, hemodynamic impact, plaque characteristics and presence of micro-emboli. Additionally, computed tomography angiography of the neck and intracranial vessels is performed to

evaluate the aortic arch and carotid anatomy. In some cases, magnetic resonance can be used as a second-level diagnostic imaging if CT is controindicated [16].

The rupture of the carotid plaque with subsequent embolism of a locally formed thrombus or plaque debris causes stroke or transient ischaemic attack, causing neurological symptoms [17].

Carotid stenosis is defined as symptomatic (SCS) when the last congruent cerebral (hemiplegia, hemiparesis) or retinal ischemic episode (transient monocular blindness or amaurosis fugax, or retinal infarction) occurred in the patient within the previous six months. However, based on reviews, the experts' panel suggests that CS should be defined as symptomatic when the last congruent cerebral or retinal ischemic episode occurred within the previous three months at most [18].

In the present work, we distinguish symptomatic CS (SCS) from severe asymptomatic CS (ACS), defined as >70% stenosis with none of the symptoms referred above.

Stenosis severity is estimated following two criteria: in the European Carotid Surgery Trial (ECST), the denominator was the estimated vessel diameter where the residual luminal diameter was measured (usually the carotid bulb); in North American Symptomatic Carotid Endarterectomy Trial (NASCET), the denominator is the diameter of a disease-free ICA segment above the stenosis, where the vessel walls were parallel. Both used minimum residual luminal diameter as the numerator [19].

For asymptomatic CS, current ESC guidelines put a threshold of 70% for formal indication. Revascularization should be discussed for symptomatic stenosis over 50% and for asymptomatic CS over 60% (Figure 1).

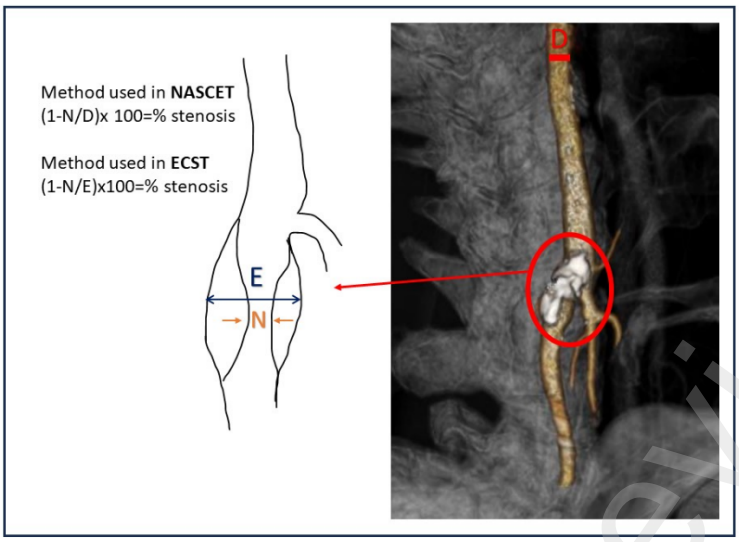

Figure 1: Schematic representation of carotid bifurcation and internal carotid artery, showing parameters to measure the degree of carotid stenosis on arterial angiography from our unit of Vascular and Endovascular Surgery; patient gave his consent for image publication.

CEA consists of surgical removal of the atherosclerotic material causing stenosis at the carotid bifurcation. It can be undertaken under local or general anaesthetic, with no difference in the outcome (Figure 2) [20].

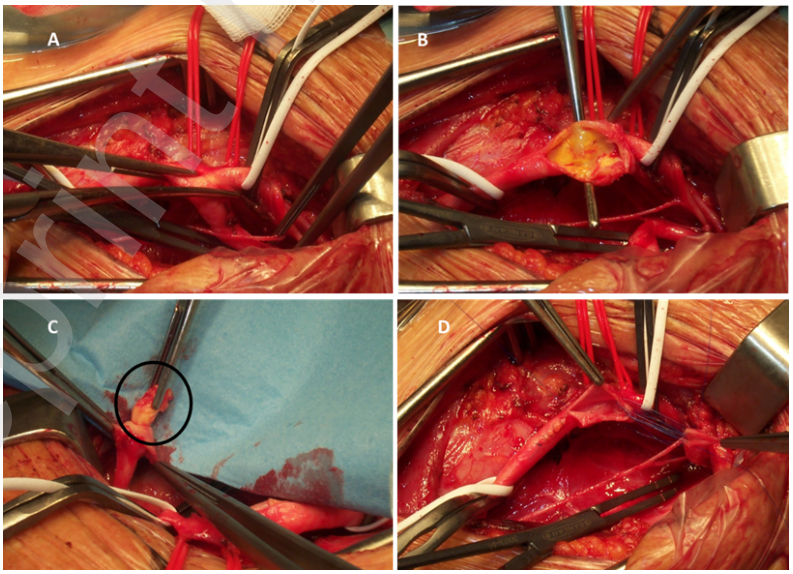

Figure 2: Intraoperative images of carotid artery endarterectomy by eversion and reimplantation. A) exposure of a carotid bifurcation with internal carotid artery and external carotid artery. B) transected internal carotid artery: the vessel is straightened and the atherosclerotic plaque visualized. C) removal of the plaque by eversion. D) transected internal carotid artery without the atherosclerotic plaque. Images are provided from our Unit of Vascular and Endovascular Surgery; patient gave his consent for image publication.

CAS is more frequently conducted under local anaesthesia. It involves passing a wire beyond the stenotic lesion and the placement of a brain protection such as the use of a filter system (to catch any pieces of atheroma dislodged during stent placement) or using balloon inflation in the external/ common carotid arteries to encourage reverse flow down the internal carotid (preventing atheromatous emboli being carried up into the intracerebral circulation), whilst the stent is placed across the stenosis and expanded to restore the normal luminal diameter (Figure 3) [16]. Early trials reported a substantial reduction in stroke risk with carotid endarterectomy in patients with severe symptomatic CS, and a modest benefit in patients with moderate symptomatic stenosis, as well as in patients with asymptomatic CS [21] (Table 1).

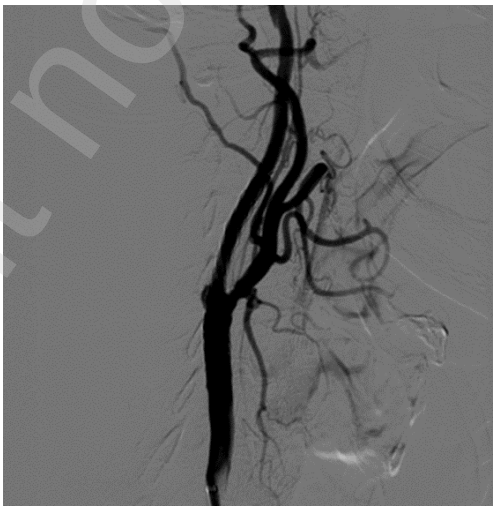

Figure 3: Completion angiography of a carotid artery stenting (CAS). Intraoperative angiography from our unit of Vascular and Endovascular Surgery; patient gave his consent for image publication.

Table 1: Indications for carotid endarterectomy and stenting according to current guidelines. Modified by Bonati et al., 2022 [17].

SYMPTOMATIC CAROTID STENOSIS		ASYMPTOMATIC CAROTID STENOSIS	
CEA	***CAS***	***CEA***	***CAS***
70-99% of stenosis: recommended. 14 days from the first ischemic event: recommended. 50-69% of stenosis: suggested. <50% of stenosis: not recommended.	Patients <70 years old: **suggested**. Patients with severe atherosclerosis of the aortic arch or tortuosity of supra-aortic vessels: **not recommended.**	60-99% of stenosis in patients at increased stroke risk: **recommended**.	Patients unsuitable for surgery: **possible options.** **Not routinely recommended**

– Abdominal Aortic Aneurysm

The abdominal aortic aneurysm (AAA) is a permanent and irreversible diameter enlargement evolving towards possible rupture. It is often associated with age (> 60 years old), smoking habits and male gender.

Elective treatment for AAA is currently indicated when it has more than 5 cm of diameter or when is less than 5 cm, but with a fast growth or peculiar morphological aspects indicative of an increased risk of breakage.

Diagnosis and prognosis of AAA relies on these imaging techniques:

Ultrasonography: This is the first line imaging tool for detection and management of small AAAs, with high sensitivity and specificity [22, 23]. Limitations are obesity or excess bowel gas, variation of aortic diameters with the cardiac cycle, the absence of serial image reconstruction to allow for stent graft planning and methodological differences (in training and instrumentation). Measurements must be performed in a plane perpendicular to the aortic longitudinal axis, which will vary in the presence of aortic tortuosity.

Computed tomography angiography (CTA): it retains several advantages for intervention planning: it provides a complete data set of the entire aorta (including the thoracic aorta) and access vessels, for post-acquisition analysis in three perpendicular planes, construction of a centerline, and accurate diameter and length measurement. This reconstruction is essential for Endovascular Aortic Repair (EVAR) pre-intervention planning and for real time peri-operative guidance. Limitations include the use of nephrotoxic contrast agents and radiation [24] (Figure 4).

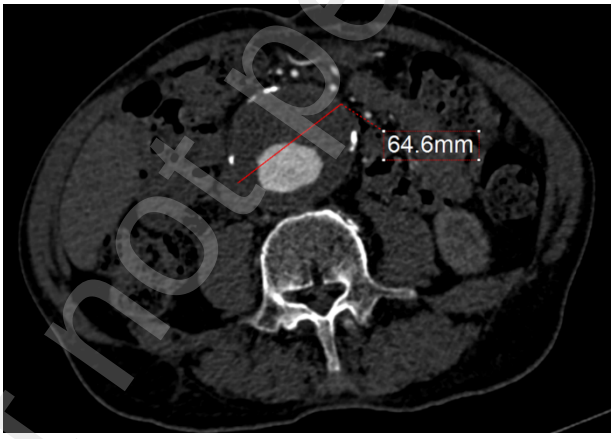

Figure 4: Pre-operative CT scan of an abdominal aortic aneurysm provided from our Unit of Vascular and Endovascular Surgery; patient gave his consent for image publication.

MRI does not require radiation or injection of iodinated contrast agents, although it requires paramagnetic contrast agent, and therefore has an advantage over CTA when AAA management requires repeated imaging [25]; however, it retains some contraindications such as claustrophobia and metal implants.

PET-CT provide clues on the presence of inflammatory cells, although the progressive increase in aneurysmal diameter is paralleled by reduction in cell density and, as consequence, on the signal intensity [24 - 27]

The surgical options for AAA are Open Surgical Repair (OSR) or Endovascular Aortic Repair (EVAR), depending on the global evaluation of the patient, comorbidities, fragility, and aortic anatomy.

OSR is performed through an incision in the abdomen. The aorta is identified at the back of the abdomen and the blood flow through the aorta temporarily stopped. Then, the AAA is replaced with a polyester (Dacron) graft. The access can be transperitoneal, the most widely used because it allows the access to all abdominal organs with relative ease, and retroperitoneal, in case of proximal aneurysm disease, inflammatory aneurysms, or in case of adhesion, due to previous interventions (hostile abdomen) (Figure 5). EVAR is carried out percutaneously. Using X-ray control a spring-loaded graft (also called stent) is passed up from the arteries in the groin into the aorta. A stent graft is meant to seal the sac from the inside of the AAA, just excluding it from the systemic circulation. Therefore, the anchoring segments need to provide both sufficient sealing and fixation.

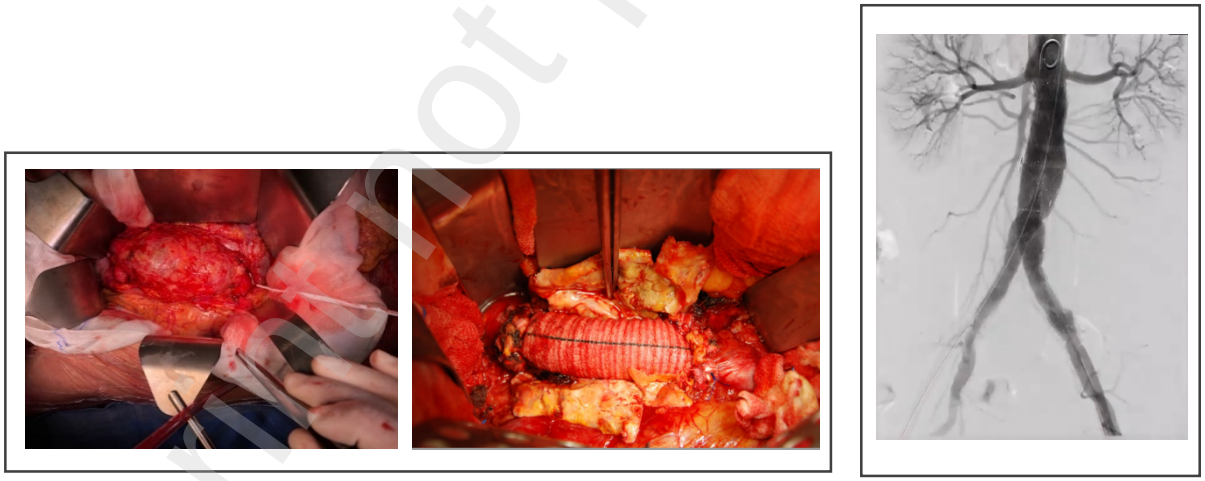

Figure 5. Open surgery: aorto-aortic graft for aneurysm repair (left panel); Intraoperative angiography of an endovascular aortic aneurysm repair (EVAR) (right panel). Images are provided by our Unit of Vascular and Endovascular surgery; patients gave their consent for image publication.

2.4 Biospecimen collection and processing

- *Peripheral Blood - derived and urine samples*

Fasting blood samples are drawn from an antecubital vein and collected in three tubes, each with a maximum volume of 6 mL (Vacuette Greiner Bio-One International GmbH). One of these tubes contains a clot activator and gel separator, designated 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 processed by centrifugation at 3500 × *g* for 15 minutes at room temperature. After centrifugation, they are stored in 500 µL aliquots. Whole blood is stored in 250 µL aliquots.

For isolation of PBMCs, 6 mL of blood is first diluted with an equal volume of saline (1:1 ratio) in 15 mL tubes. Density gradient centrifugation is then performed over 3 mL of Lympholyte separation medium (Cedarlane, Canada) at 1800 × *g* for 20 minutes without a brake. The interface ring, which contains PBMCs, is carefully recovered and rinsed with saline through centrifugation. The resulting pellet is divided into four aliquots, subjected to a brief high-speed centrifugation (30 seconds), and subsequently dry stored at -80°C.

Urine samples are exclusively collected from patients diagnosed with AAA. These samples are carefully stored in six vials containing 1 mL of urine within monitored and alarmed facilities at -80°C.

- *Tissue sample*

After surgical excision, tissue samples are placed in a dry, sterile, disposable container and transported from the operating room to the BioVasc Lab. Depending on the size and quality of the tissue, it is processed in consecutive segments. Typically, two aliquots of approximately 0.5 to 1 gram each are immediately stored at -80°C for molecular analysis.

The remaining segments are fixed in 10% formalin at room temperature and then sent to the Anatomy Pathology Unit, where they undergo processing following standard protocols. In brief, all specimens are immersed in 10% buffered formalin for an overnight period (approximately 12-18 hours).

Subsequently, they are routinely processed and embedded in paraffin to produce histologic slides measuring 3-5 µm in thickness, which are then stained with hematoxylin/eosin (Figure 6).

Biopsies from CS comprehend the plaque, with core and shoulder, and a portion of medial layer retrieved with the lesion. CS plaque retains a heterogeneous composition, with different recruitment of inflammatory cell populations, depending on the blood flow direction and portion of the lesion (shoulder vs. core). Considering this variability, aliquots are picked up as represented in Figure 7.

Biopsies from AAA are often more abundant; therefore, fragments represent the histology of the same lesion.

After the completion of the standard diagnostic procedure, the paraffin blocks are carefully archived at room temperature, stored in cardboard boxes that are shielded from dust, light, and heat sources. Beginning in 2022, one tissue segment is also included in OCT and stored at -80°C.

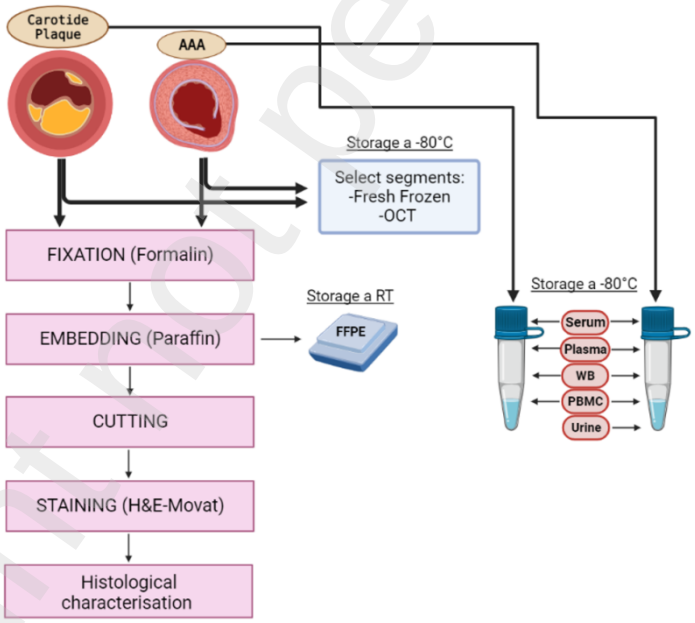

Figure 6: Flowchart processing of vascular sample before and after surgery. Carotid plaque: atherosclerotic lesion from patients with high/moderate-graded carotid symptomatic stenosis or with d carotid asymptomatic stenosis; AAA: aortic wall from patients with abdominal aortic aneurysm; H&E: hematoxylin- eosin staining; FFPE: Formalin-Fixed Paraffin-Embedded; OCT: Optimal

cutting temperature used to embed tissue samples prior to frozen sectioning on a microtome-cryostat; WB: Whole Blood; PBMCs: peripheral blood mononuclear cell.

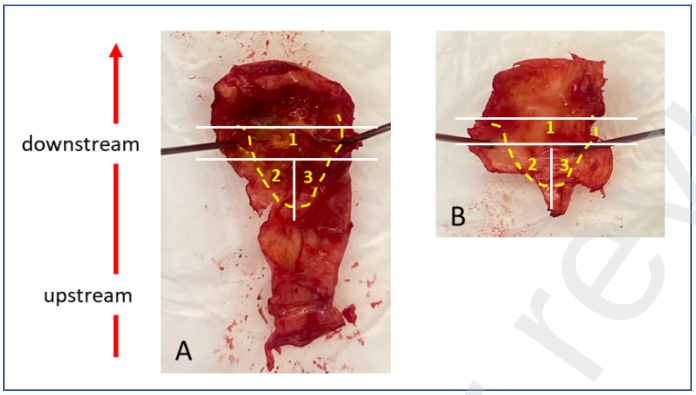

Figure 7: Criteria for CS dissection and storage. When recognizable, the lesion is oriented according to the bloodstream: section 1, comprehending the core (in the middle) and the shoulder (at both sides), is formalin fixed-paraffin embedded (FFPE); sections 2 and 3, comprehending mostly plaque shoulder and, at a lesser extent, the core of the upstream portion of the plaque, are stored at -80°C. Often, the arterial wall (A) and the inner side containing the plaque, (B) are divided into complementary fragments.

- *Quality control tests on biological specimens*

When required, the RNA integrity number (RIN) of dry-frozen PBMCs is assessed using the Agilent 2100 Bioanalyzer in combination with the RNA 6000 Nano Kit (Agilent Technologies, Waldbronn, Germany). This assessment is carried out in strict accordance with the manufacturer's provided instructions.

For all Formalin-Fixed Paraffin-Embedded (FFPE) segments, we prepare consecutive sections measuring 3-5 µm in thickness. These sections are then stained with both haematoxylin-eosin (HE) and MOVAT to evaluate tissue morphology.

- *Clinical data collection and management*

Each sample collected in the GTB-VD is associated with clinical data corresponding to the donor patient. Until 2021, the collection of clinical data consisted in the storage of physical copies of patient clinical records. Starting from January 2021, the clinical data is being collected in the prospective electronic database system of the Italian Society of Vascular and Endovascular Surgery (https://www.sicvereg.it/). Clinicians registered all available patient data in the electronic database system, thus enabling calculations and computations of clinical data associated with the biobank samples.

The electronic database system does not include patient names or any patient information that could be used to identify a patient. Patients are registered under a study number that is electronically assigned by a designated tool. This study number is used during data collection and data processing.

3. RESULTS

3.1 Characterization of the Study populations

The GTB-VD serves as a prospective observational biobank and databank specifically focused on patients with AAA and CS diseases. The GTB-VD has been accumulating biological samples from patients who have undergone surgery for CS and AAA at the Vascular and Endovascular Surgery Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy, since November 2018. Thus far, 537 subjects have been recruited; Table 2 provides an overview of the number of cases of which a standardized and comprehensive collection of biological samples is available.

Table 2: Number of patients enrolled in the biobank since 2018 until the end of July 2023.

Type of surgical Procedure	*Number of Patients*
CAS	40
CEA	311
EVAR	117
OSR	69
Total	**537**

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

While our efforts in data collection have been significantly enhanced since 2021, samples recovered prior to this date are by no means lacking in clinical information. These earlier samples are still associated with fundamental clinical data, providing valuable insights into the patients' demographics and essential medical characteristics. With the advent of the SICVEREG data collection initiated in 2021. Table 3 presents an overview of these demographic and clinical attributes in respect to the pathology, further depicted in table 4 as divided for type of surgery.

Table 3: Demographic and clinical characteristics of subjects enrolled in the biobank.

Variable	AAA (n = 186)	CS (n = 351)	*p* value
Males(%)	167 (89.8)	238 (67.8)	<0.001
Age (median [IQR])	77.00 [71.00, 82.00]	76.00 [70.00, 81.00]	0.075
Diabetes(%)	21 (11.3)	91 (25.9)	<0.001
Pulmonary History(%)	31 (16.7)	22 (6.3)	<0.001
Cardiac History(%)	32 (17.2)	54 (15.4)	0.672
Dyslipidemia(%)	64 (34.4)	153 (43.6)	0.049
Hypertension History(%)	118 (63.4)	233 (66.4)	0.558
Peripheral artery disease(%)	5 (2.7)	36 (10.3)	0.003
Leukocytes x10E9/L (median [IQR])	6.90 [5.89, 8.24]	7.40 [6.30, 8.75]	0.018
Erythrocytes x10^{12}/L (median [IQR])	4.60 [4.20, 4.90]	4.40 [4.10, 4.80]	0.003
Hemoglobin g/L (median [IQR])	140.00 [127.00, 149.00]	130.00 [119.50, 143.00]	<0.001
Platelets x10^{9}/L (median [IQR])	195.00 [167.50, 232.50]	215.00 [178.50, 258.00]	0.002
Neutrophils x10^{9}/L (median [IQR])	4.46 [3.72, 5.30]	4.68 [3.86, 5.93]	0.137
Lymphocytes x10^{9}/L (median [IQR])	1.49 [1.18, 1.91]	1.65 [1.26, 2.12]	0.082
Creatinine mg/dL (median [IQR])	1.00 [0.90, 1.20]	1.00 [0.80, 1.30]	0.182
eGFR mL/m^2/1,73mq (median [IQR])	70.00 [55.00, 83.00]	72.50 [50.00, 86.00]	0.711
Stage 1 of CKD(%)	19 (10.2)	46 (13.1)	0.402
Stage 2 of CKD(%)	91 (48.9)	122 (34.8)	0.002
Stage 3a of CKD(%)	36 (19.4)	42 (12.0)	0.029
Stage 3b of CKD(%)	11 (5.9)	36 (10.3)	0.125
Stage 4 of CKD(%)	8 (4.3)	5 (1.4)	0.077
Stage 5 of CKD(%)	0 (0.0)	5 (1.4)	0.245

eGFR estimated glomerular filtration rate; CKD, Chronic Kidney Disease; 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.

Table 4: Patient demographics and comorbidities of the study population, as divided for type of surgery.

Variable	CAS (n = 40)	CEA (n = 311)	EVAR (n = 117)	OSR (n = 69)
Males(%)	28 (70.0)	210 (67.5)	106 (90.6)	61 (88.4)
Age (median [IQR])	76.00 [68.00, 81.00]	76.00 [70.00, 81.00]	79.00 [73.00, 83.00]	75.00 [68.00, 79.00]
Diabetes(%)	10 (25.0)	81 (26.0)	13 (11.1)	8 (11.6)
Pulmonary History(%)	1 (2.5)	21 (6.8)	19 (16.2)	12 (17.4)
Cardiac History(%)	7 (17.5)	47 (15.1)	25 (21.4)	7 (10.1)
Dyslipidemia(%)	11 (27.5)	142 (45.7)	32 (27.4)	32 (46.4)
Hypertension History(%)	24 (60.0)	209 (67.2)	74 (63.2)	44 (63.8)
Peripheral artery disease(%)	2 (5.0)	34 (10.9)	3 (2.6)	2 (2.9)
Leukocytes x10^9/L (median [IQR])	7.52 [6.54, 9.04]	7.40 [6.21, 8.73]	6.70 [5.72, 8.17]	7.18 [6.38, 8.3
Erythrocytes x10^{12}/L (median [IQR])	4.50 [4.00, 4.80]	4.40 [4.10, 4.80]	4.55 [4.10, 4.90]	4.70 [4.50, 5.0
Hemoglobin g/L (median [IQR])	129.00 [117.50, 138.00]	130.00 [120.00, 144.00]	134.00 [124.00, 147.50]	144.50 [135.0 150.25]
Platelets x10^9/L (median [IQR])	216.00 [185.00, 258.00]	215.00 [177.50, 258.00]	190.00 [164.50, 223.00]	209.00 [181.7 242.75]

Neutrophils x10^9/L (median [IQR])	4.97 [4.14, 6.46]	4.65 [3.80, 5.80]	4.41 [3.78, 5.35]	4.68 [3.47, 5.2
Lymphocytes x10^9/L (median [IQR])	1.65 [1.13, 2.03]	1.65 [1.27, 2.14]	1.41 [1.15, 1.77]	1.63 [1.27, 2.0
Creatinine mg/dL (median [IQR])	1.10 [0.90, 1.20]	1.00 [0.80, 1.30]	1.00 [0.90, 1.20]	1.00 [0.80, 1.2
eGFR mL/m^2/1,73mq (median [IQR])	64.00 [56.50, 79.75]	74.00 [50.00, 86.75]	69.00 [53.00, 82.00]	74.00 [55.75, 88.25]
Stage 1 of CKD(%)	4 (10.0)	42 (13.5)	8 (6.8)	11 (15.9)
Stage 2 of CKD(%)	23 (57.5)	99 (31.8)	65 (55.6)	26 (37.7)
Stage 3a of CKD(%)	5 (12.5)	37 (11.9)	22 (18.8)	14 (20.3)
Stage 3b of CKD(%)	5 (12.5)	31 (10.0)	9 (7.7)	2 (2.9)
Stage 4 of CKD(%)	1 (2.5)	4 (1.3)	5 (4.3)	3 (4.3)
Stage 5 of CKD(%)	0 (0.0)	5 (1.6)	0 (0.0)	0 (0.0)

eGFR, estimated glomerular filtration rate; CKD, Chronic Kidney Disease ; 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.

These insights provide an essential understanding of the biobank's patient populations and are indispensable to our mission of identifying molecular targets and circulating biomarkers associated with atherosclerotic complications in vascular diseases. Notably, our biobank encompasses a broad spectrum of ages, with most subjects being over sixties and more advanced ages. Table 5 and Table 6 show the distribution of the carotid stenosis cohort patients by age and by type of carotid stenosis, symptomatic and asymptomatic, and the distribution of the AAA cohort patients. This insight provides an understanding of the patient populations. This demographic diversity adds depth to our research, serving as a valuable resource for characterizing our patient cohorts.

Table 5: Number of patients enrolled in the for Carotid Endarterectomy and Carotid Artery Stenting procedures.

	CEA (n = 314)				CAS (n = 42)			
Age (Years)	**Male (n = 213)**		**Female (n = 101)**		**Male (n = 31)**		**Female (n = 11)**	
	Symptomatic (n = 54)	Asymptomatic (n = 159)	Symptomatic (n = 16)	Asymptomatic (n = 85)	Symptomatic (n = 6)	Asymptomatic (n = 25)	Symptomatic (n = 1)	Asymptomatic (n = 10)
≤ 50	0	0	0	3	0	0		0
51-60	3	9	2	6	1	2		2
61-70	13	47	4	19	3	2		3
71-80	27	63	6	8	1	14	1	4
81-90	11	40	4	18	1	7		1
≥ 91	0	0	0	1	0	0		0

CAS, carotid artery stenting; CEA, carotid endarterectomy.

Table 6: Patient age distribution in EVAR and OSR surgery cohorts by gender.

Age (years)	**EVAR (n = 230)**		**OSR (n = 138)**	
	Male (n = 208)	**Female (n = 22)**	**Male (n =124)**	**Female (n = 14)**
≤50	0	0	0	0
51-60	3	1	2	0
61-70	18	1	21	2
71-80	42	4	34	3
81-90	36	5	5	2
≥91	5	0	0	0
TOT	104	11	62	7

EVAR, endovascular repair; OSR, open surgical repair.

In the recent years, with the advent of endovascular techniques, the landscape of vascular surgery has witnessed a shift toward minimally invasive procedures. As represented in figure 8, the plot illustrates the volumes of AAA interventions/year since 2015 at our Unit, highlighting how endovascular interventions have surged in number. However, it is essential to note that open surgical repairs still hold their place in clinical practice, allowing us to obtain tissue samples from aneurysms during open repairs. This dynamic interplay between traditional open surgeries and emerging endovascular approaches underscores the complexity and depth of our biobank's data, further enriching our research efforts and enabling retrospective, long-term evaluations on the different surgical approaches.

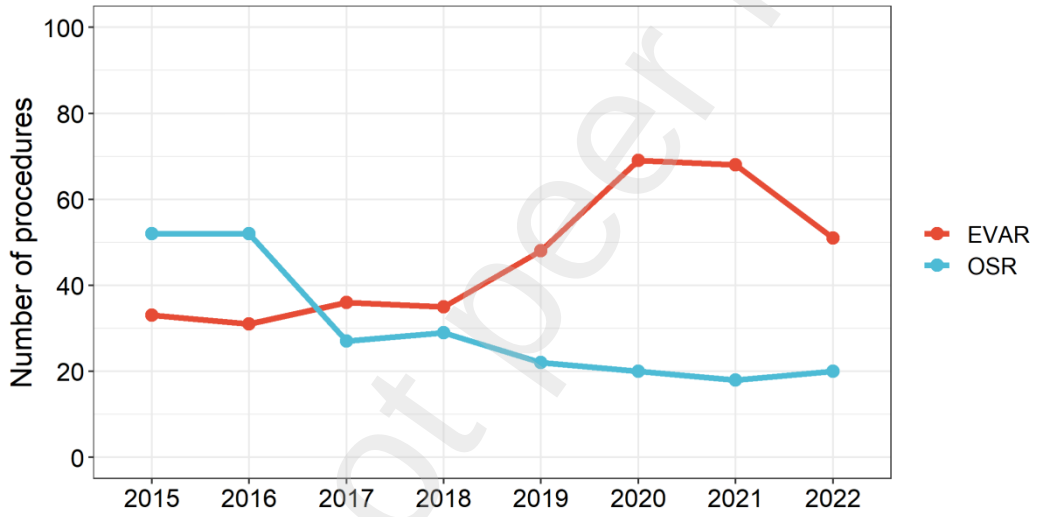

Figure 8: Volume of endovascular aortic repair procedures and open surgical aortic repair since 2015 until 2022.

4. DISCUSSION

4.1 BIOBANKS PURPOSES AND FEATURES

The harmonization and standardization of biobanks, where biological specimens and associated data coexist [28], have assumed paramount importance in the era of -omics sciences and systems medicine. This harmonization marks a significant shift from reactive personalized care, which relies on responses to observed failures, to a precision-based approach.

Precision medicine, which integrates patients' genetic variations, environmental factors, and lifestyles, aims to provide effective medical technologies to all patients. Its goal is to identify the best therapies for each individual, taking in account factors such as efficacy and cost while minimizing the risk of side effects.

Biobanks represent a collaborative effort involving healthcare practitioners, researchers, and bioinformaticians, particularly in the study of complex diseases. While historically, biobanks originated as decentralized collections for isolated research projects, they now adhere to standardized parameters for sample harvesting, processing, and storage [29].

According to the European Commission Joint Research Centre, modern biobanks exhibit key characteristics, including the collection and storage of biological materials linked to medical and epidemiological data, facilitating dynamic, continuous, and long-term collection. Moreover, they seamlessly integrate with ongoing research, prioritize donor privacy through pseudonymization, and uphold governance standards for the future use of samples in diverse research projects [30]

Several circumstances occurring before biospecimen collection can affect the reliability of analytical results. Conditions of the subject (genotype, lifestyle, nutrition, medication, concomitant diseases, surgical interventions, etc.), are of great importance. Most of these variables cannot be standardized; nonetheless, they deserve to be recorded in the associated database. The collection phase of biological material begins when the biobank’s staff receive samples. From then on, the state of the samples is influenced by transportation (compliance with the cold chain regime, duration), chain of custody in the

laboratory and procedures for isolating fractions for subsequent research. Finally, the registering and exhaustive annotations of samples in the database enables the proper matching of information. These critical points can influence the total outcome of studies [31].

Human biological samples encompass a wide range of materials, necessitating standardized practices for pre-analytical steps. Blood, a commonly used biospecimen, requires careful consideration of anticoagulants to minimize biases in data collection. While fresh or frozen tissues often yield higher-quality DNA and RNA, technologies have adapted to test formalin-fixed, paraffin-embedded (FFPE) samples at room temperature, considering the prevalence of FFPE tissues in biobanks [31].

The publication of ISO 20387:2018 'Biobanking—General requirements for biobanking' provides essential guidelines for the organization and processing of biological samples, ensuring minimum standardization requirements for the reproducibility and comparability of scientific research results. Ethical considerations, such as informed consent, sample ownership, confidentiality protection, veto rights, and biobank sustainability, are also addressed [32, 33].

4.2 GTB-VD: OUR EXPERIENCE

Consistently with these main features, our experience of biobanking has been built up starting from isolated studies on atherosclerosis, with a prominent focus on carotid artery stenosis (CS) and abdominal aortic aneurysm (AAA).

Atherosclerosis is a complex, systemic and multifaceted disease. Borrowing an assumption from the experience on acute coronary syndrome [34], the complexity of atherosclerotic major events, as occurs also for ischemic stroke and abdominal aortic rupture, surpasses the traditional clinical categorization, so far based on ultrasound/CT image analysis, that oversimplifies a spectrum of possible manifestations and obscures mechanisms that may require different strategies of protection. Furthermore, this statistics-based criterion exposes to surgical procedures even subjects who would never encounter complications; by other side, it does not permit an early detection of those with a fast and ominous progression. Both

CS and AAA still lack biomarkers to formulate precise prognosis and therapeutic molecular targets to reverse or just counteract the disease progression.

In the last 15 years, our research efforts in CS have unveiled crucial insights, such as the association between high serum lipoprotein(a) levels and acute coronary syndrome in severe carotid stenosis patients [9].

A milestone in the study of the carotid stenosis has been added in 2010, with the observation that systemic and intraplaque inflammation, together, could influence global patient vulnerability for ischemic stroke; importantly, has pointed out that "orientation matters", as the upstream or downstream portions of the plaque differently contribute to its vulnerability [35]. Further observations recognized a protective role for Vitamin D receptor, being an active anti-inflammatory mediator within atherosclerotic plaques [36], a predictive value of serum and intraplaque c-reactive protein (CRP) in patients with severe CS for major cardiovascular events at an 18-month follow-up period [10], and an immunoregulatory role of resistin in inhibiting neutrophil-mediated atherosclerotic activities within the plaque [11]. Lately, it has been shown that also ficolins may have a predictive value of cardiovascular disease symptoms, as they correlate with local and systemic inflammation, ultimately leading to plaque vulnerability [12].

AAA is a chronic aortic dilation potentially leading to rupture. It is associated with high morbidity/mortality, being responsible for 11.000 deaths/y in USA [37]. Fifty percent of patients with aortic rupture die outside hospital, 30–40% have peri- and post-operative complications. The Screening Abdominal Aortic Aneurysm Genoa (S.A.Ge) study showed the importance of early screening program for AAA in 65- to 75-year-old men like a cost-effective strategy [38]. The diagnosis through different imaging modalities assessing aneurysm size became quite relevant.

In previous studies, we observed that AAA patients display specific pattern of monocyte subset distribution, with an increased profibrotic profile, indicating a systemic involvement of the immune response [4, 5]. Moreover, AAA is often associated with anemia, a neglected mild-to-moderate renal failure and cardiorenal syndrome [6]. This finding prompted us to collect also urine samples from AAA

patients to assess urinary biomarkers of renal functions, to provide an accurate profile for surgical risk stratification and suggestion for the more adequate type of intervention and imaging, i.e., adopting CO_2 for angiography instead of nephrotoxic contrast agents.

Since the 2018 on, when the GTB-VD has been established, more than 537 cases have been recruited; of them, 34.6% underwent to surgery for AAA repair and 65.4% for carotid stenosis. About 68% of CS and 90% of AAA patients are males. The prevalence of diabetes and peripheral artery diseases are significantly higher in CS patients, while pulmonary history (referred as BPCO, emphisema) and a moderate renal function impairment, as evidenced by the prevalence of chronic kidney disease (CKD) stage 3, is more frequent among AAA subjects.

Looking at the surgical technique in AAA repair, 117 out of 186 cases are EVAR, indicating that, over time, the endovascular approach has become the most adopted, on the basis of the need of the population. Our Unit represents a highly specialized center (regional hub for complex aortic surgery) of the University hospital Policlinico San Martino IRCCS, and provide health assistance for the population of Genoa and neighboring areas, characterized by the increased older classes compared to the national average (12.7% vs. 11.1 % in the 65 - 74 years old age group and 15.8% vs. 11.7 % for over 75, respectively) (https://www.istat.it/it/file//2020/05/07-Liguria-Scheda).

Currently, GTB-VD is fueling projects to evaluate cell stress response pathways in AAA and CS respectively. Namely, redox balance, inflammation and possible association with circulating/urinary biomarkers are taken in consideration; the final goal is to identify underlying mechanisms, molecular target and systemic biomarkers for optimizing new strategies of risk stratification and conceive new therapeutic options to counteract major complications.

To this purpose, the contribution of GTB - VD in bioengineering research and application is mandatory: GTB - VD facilitates the identification of biomarkers associated with vascular diseases; these biomarkers can serve as tools to design targeted approaches such as nanosystems and biosensors, both for diagnosis and treatment.

Furthermore, vascular tissues and cells could serve for tissue engineering and regenerative medicine studies. Indeed, to have a complete validation of scaffolds, they must be tested *in vitro* with blood, according to ISO 10993.

In the context of vascular drug delivery, our experience relies on hemocompatibility studies by using blood provided by the GTB from healthy donors to prove the hemocompatibility of nanoliposomes [39] and of antibody-decorated polymeric nanoparticles [40].

Collaborating with the Laboratory of Clinical and Experimental Vascular Biology, the Unit of Anatomic Pathology, and the Biological Resource Center, we are part of the biobank network of the non-profit Association for the Study of Cardiovascular Diseases "Rete Cardiologica". This collaborative network streamlines biobanking activities across 20 Scientific Institutes of Hospitalization and Care (IRCCS) in Italy, adhering to shared procedures and ethical guidelines outlined in ISO 20387, with the goal to develop high consistency research programs in cardiovascular diseases, not reachable by individual biobanks.

In the era of precision medicine, biobanks serve as essential resources, relying on the generosity of individuals who contribute their samples for research and long-term preservation. We welcome collaborative proposals and sample contributions to our biobank, recognizing their pivotal role in advancing medical knowledge and improving patient care.

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Declarations

Ethics Statement: Document no. 1719, formally signed at our Institution IRCCS Policlinico San Martino, on 30 November 2018, approves the policy and rules of the GTB-VD. GTB-VD is an integral part of the institutional Core facility CRB, Centro di Risorse Biologiche and participates in the national node (BBMRI.it) of the European infrastructure BBMRI -ERIC (Biobancking and Biomolecular Resources, Research

Infrastructure, https://urlsand.esvalabs.com/?u=https% 3A%2F%2Fdirectory.bbmri.it%2F%23%2Fboard&e=996425d6&h =1e42624d&f=n&p=y)

Conflicts of Interest: The authors declare no conflict of interest.

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