IJAE

Italian Journal of Anatomy and Embryology

Official Organ of the Italian Society
of Anatomy and Histology

Vol. 128
N. 2
2024
ISSN 1122-6714

FIRENZE
UNIVERSITY
PRESS

IJAE
Italian Journal of Anatomy and Embryology
Official Organ of the Italian Society of Anatomy and Histology

Founded by Giulio Chiarugi in 1901

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Italian Journal of Anatomy and Embryology

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Vol. 128, n. 2 - 2024

Firenze University Press

Italian Journal of Anatomy and Embryology
https://www.fupress.com/ijae
ISSN 1122-6714 (print) | ISSN 2038-5129 (online)

Direttore responsabile: **Ferdinando Paternostro, University of Firenze, Italy**
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Published by Firenze University Press

Firenze University Press
Università degli Studi di Firenze
via Cittadella, 7, 50144 Firenze, Italy
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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
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Citation: Sassoli, C., Tani, A., Chellini, F., Nosi, D., & Zecchi-Orlandini, S. (2024). In memory of Lucia Formigli on the 10th anniversary of her death: the scientist, the teacher, the woman. *Italian Journal of Anatomy and Embryology* 128(2):3-4. https://doi.org/10.36253/ijae-15552

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

In memory of Lucia Formigli on the 10th anniversary of her death: the scientist, the teacher, the woman

Chiara Sassoli, Alessia Tani, Flaminia Chellini, Daniele Nosi, Sandra Zecchi-Orlandini

Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, Imaging Platform, University of Florence, Florence, Italy

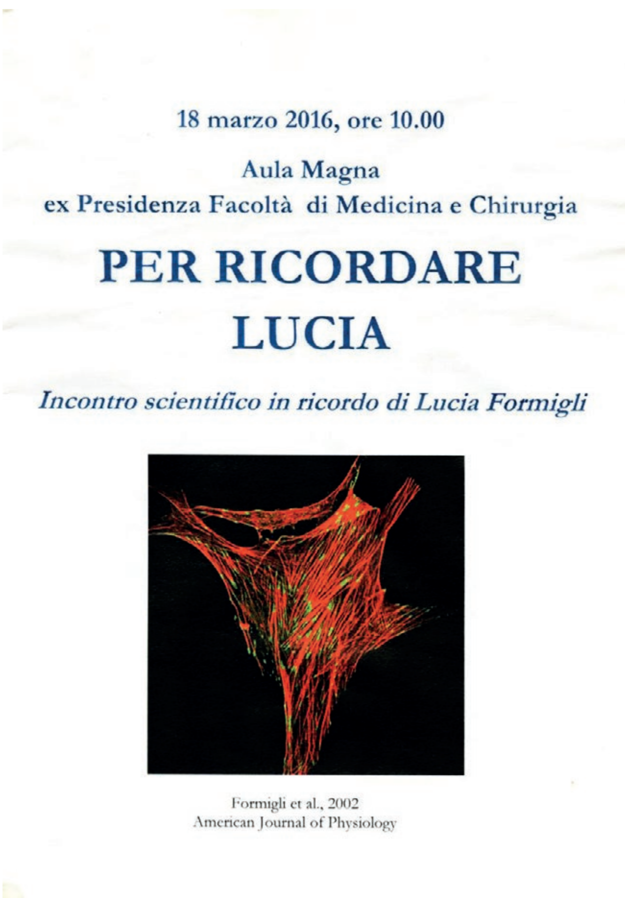
Ten years ago, on March 18th, 2014 in Florence Prof. Lucia Formigli, 54 years old, passed away. We, who had the privilege and the pleasure of working with her, an extraordinary person with many talents, wish to pay her a remembrance.

She graduated in Biological Science at the University of Florence in September 1987. From 1988 to 1990 she was a Visiting Fellow at the School of Medicine of the University of Nevada- Reno, USA under the supervision of Dr Gregory J. Highison. From 1990 to 1992 she was recipient of a Research Fellowship and from 1992 to 2004, she was Researcher of Human Anatomy at the Department of Anatomy, Histology and Forensic Medicine, Faculty of Pharmacy of the University of Florence. From 2004 to 2014 she was asked as Associate Professor of Human Anatomy by the Department of Anatomy, Histology and Forensic Medicine, subsequently Department of Experimental and Clinical Medicine of the University of Florence. She was a member of the Italian Society of Anatomy and Histology.

Prof. Lucia Formigli was a brilliant and passionate scientist capable of transmitting skills, passion and enthusiasm. A serious, honest, restless,

ISSN 1122-6714 (print) | ISSN 2038-5129 (online) | DOI: 10.36253/ijae-15552

determined, curious, optimistic and extremely generous researcher, a mentor and an example for her students and colleagues. Her scientific interests were mainly aimed to depict the cellular and molecular mechanisms underpinning tissue repair and regeneration with a focus on bone, cardiac and skeletal muscle. She always wanted to be updated on scientific trends thus contributing to insert in the research the most cutting-edge imaging technologies including confocal laser scanning microscopy, two-photon excitation microscopy, atomic force microscopy and living imaging. In all her numerous outstanding studies, although morphology was the main actor, she included the physiological investigations firmly convinced, as she was, that a multidisciplinary approach was the real basis of science and that "*structure without function is a corpse; function without structure is a ghost*" (Vogel S. and Wainwright S.A., Addison-Wesley Publishing Company, 1969). Moreover, her enthusiasm, energy and curiosity resulted in highly prolific collaborations with histologists, biochemists, pharmacologists, physicists, surgeons, endocrinologists, anesthetists, oncologists and odontologists. Prof. Lucia Formigli had the ability to never give up and to turn any result, even the unexpected ones, into new avenues and promising perspectives for research. She was co-author of more than 100 peer-reviewed articles and invited reviews in the field of bone remodeling, cardiac repair/regeneration and skeletal muscle differentiation. In the same field she was invited at both national and international meetings and workshops bringing her own personal contribution. She was a natural leader coaching many PhD students and young researchers who joined the studies, then becoming lifelong friends.



Flier of the scientific meeting organized to remember Prof. Lucia Formigli on March 18th, 2016, two years after her death.

Two years after her death our group decided to honor her memory by organizing a scientific meeting with the participation of colleagues from different Universities and young researchers. On that occasion, the main hall of the Department was crowded with friends, students and coworkers, with emotional scientific presentations related to the main research topics of Lucia.

Prof. Lucia Formigli carried out didactic activities for many years in the Pharmacy degree course at the University of Florence. She was a much appreciated and beloved teacher of Human Anatomy due to her competence, availability and personality, able to involve the students, catch their attention, listen to them, being never indifferent to their special needs. She was co-author of textbooks in the field of macroscopic and microscopic Human Anatomy with wide national diffusion.

Prof. Lucia Formigli was capable of building and aggregating. The same kind of devotion and care she applied to scientific research and teaching was also applied to the relationship with people she was interacting with and this was one of her best qualities. Indeed, the harmony in the research group was an irrevocable priority.

We will never forget her and try to transmit her teaching and example to the young generations.

IJAE
ITALIAN JOURNAL OF
ANATOMY AND EMBRYOLOGY

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Citation: Della Rocca, Y., Diomede, F., Mazzone, A., Trubiani, O., Pizzi-Canella, J., & Marconi, G.D. (2024). Performance evaluation in titanium implant surface: A literature review. *Italian Journal of Anatomy and Embryology* 128(2): 5-12. https://doi.org/10.36253/ijae-15320

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Review

Performance evaluation in titanium implant surface: A literature review

Ylenia Della Rocca[1,*], Francesca Diomede[1], Antonella Mazzone[1], Oriana Trubiani[1] , Jacopo Pizzicanella[2,§], Guya Diletta Marconi[1,§]

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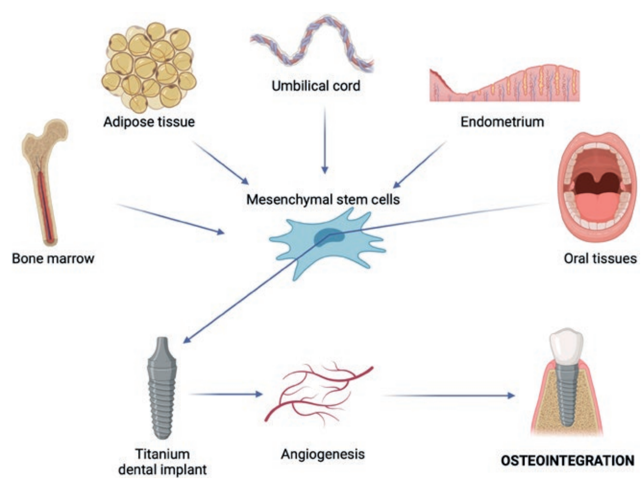
Abstract. The development of biomaterials is a constantly evolving. In the last decades, several biomaterials have been developed in various applications which have led to important advances in the processing of materials in medical fields such as orthopedics, dentistry, as well as in tissue engineering. Titanium is one of the most widely used biomaterials for dental implants due to its good mechanical properties and its excellent biocompatibility. The surface of titanium implants can be treated with various methods that improve the implant osteointegration. A better implant integration is possible also due to the interaction between titanium and oral cavity mesenchymal stem cells (oral MSCs) allowing an increase of osteogenic differentiation and release of matrix components. Furthermore, the interaction between titanium with certain surface treatments and oral MSCs leads to an increase in vascularization, fundamental for osseointegration. This review aims to analyze titanium as a dental implant biomaterial in the wound healing and osteointegration processes.

Keywords: dental implant, osteointegration, vascularization, oral stem cells, titanium implants.



ISSN 1122-6714 (print) | ISSN 2038-5129 (online) | DOI: 10.36253/ijae-15320

1. INTRODUCTION

The term “biomaterial” refers to natural or synthetic materials that are placed in contact with living tissues and / or biological fluids (1). The development of biomaterials is a constantly evolving scientific field that began thousands of years ago since prehistoric times through the experimentation of natural remedies for the treatment of diseases and the preservation of health. These remedies were essentially based on the understanding of the beneficial properties of the available materials and studies of procedures to transform them into tools for medical applications. The gradual evolution of materials has produced biomaterials with more efficient performance and superior versatility and reproducibility (2). In the last decades, several biomaterials have been developed in various applications which have led to important advances in the processing of materials in medical fields such as orthopedics, dentistry, as well as in tissue engineering (3). Directly associated with the term biomaterial is the definition of ‘biocompatibility’ since to define a material as biomaterial it must be biocompatible. “Biocompatibility” is now only loosely defined as “the ability of a material to function with adequate host response in a specific application”. Biocompatible biomaterials should be chemically inert, hypoallergenic, non-carcinogenic, without negative influences on the biological system (4). In particular, since the biocompatibility of dental materials is of fundamental clinical interest, it has acquired growing interest in research in recent years (1). Research on the development of different types of materials for dental applications is continuously improving for oral health preservation (5). Biomaterials can be classified according to their chemical structure, distinguishing in metals, ceramics, polymers, and composites, or according to their degree of interaction with the surrounding biological environment: inert, bioactive, bioabsorbable. Another type of classification is based on the origin depending on whether it is of synthetic or natural production (6). One of the most widely used biomaterials in implants is titanium, which was first introduced as a dental implant by Brånemark in the late 1970s (7). Titanium and Ti alloys have biocompatibility and good mechanical properties. However, osseointegration of the Ti implant surface with new bone is slow. Since the osseointegration process depends not only on the implant material, but also on other characteristics such as the implant surface, different implant surface modification techniques are tested to accelerate the osseointegration (8). Furthermore, the interaction of titanium with mesenchymal stem cells (MSCs) also affects the process of osseointegration (9). Bone degradation and formation is enabled by the differentiation of MSCs into specialized cells such as osteoclasts and osteoblasts. The extracellular matrix (ECM), containing proteins such as collagen (especially type I), fibronectin, laminin and proteoglycans, interacts with resident cells influencing their behavior. The ECM, by modulating growth factors, could support the self-renewal capacity and differentiation of MSCs. The interaction between cells and the ECM is critical for maintaining tissue homeostasis (10). Furthermore certain titanium implants in contact with the MSCs determine the process of osseointegration through the induction of angiogenesis (11). An important role in osseointegration seems to be played by the interaction between the titanium of the implant and the extracellular vesicles (EVs). EVs are cell derivatives delimited by a membrane that contain materials of different nature (proteins, nucleic acids, lipids) and are involved in intercellular communication. EVs include exosomes, microvesicles/microparticles induced by activation or apoptosis, and apoptotic bodies. Current research interest in the field mainly focuses on two main types of EVs: exosomes and microvesicles (12). Exosomes could promote angiogenesis and upregulate the expression of genes related to osteogenic differentiation through miRNAs (13). The present review analyzes the titanium surface modifications in dental implant material and its interaction with MSCs and EVs for the induction of angiogenesis and osseointegration processes.

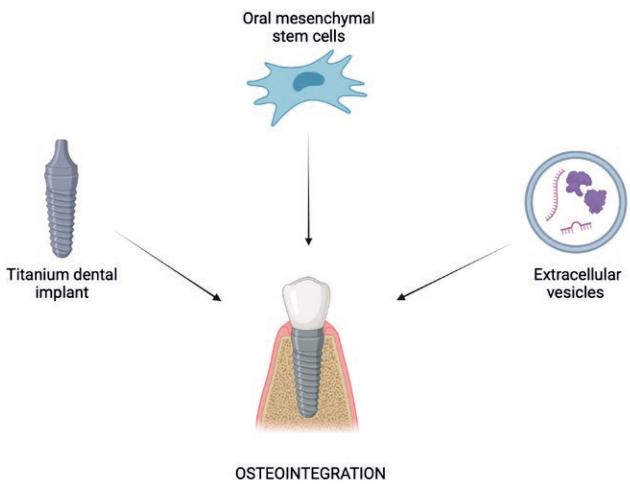


Figure 1. Interaction between titanium dental implant, mesenchymal stem cells (MSCs) and extracellular vesicles (EVs) that leads to osteointegration. (Created with BioRender.com).

2. TITANIUM IN DENTAL IMPLANTS

Titanium is considered one of the best biological metal material (14). The wide use of titanium is mainly due to its good mechanical properties, high corrosion resistance and its excellent biocompatibility. Titanium is used for many dental applications, as well as for various dental instruments such as orthodontic wires, endodontic files, dental implants and cast restorations (15). The surface characteristics of titanium implants appear to be particularly relevant for the establishment of the osseointegration process and bone remodeling at the boneimplant surface level (16). The surface structure of titanium dental implants can in fact modulate the activity of MSCs leading to upregulation of genes related to the formation of osteoblasts and the release of components of the ECM, that is the first step that leads to the early stage of bone formation (17). The surface topography of the titanium implant regulates the cellular response and therefore represents one of the main factors influencing the success of a dental implant (18, 19). There are different treatments that generate different titanium implant surfaces leading to more or less rapid bone-titanium integration. Sandblasted/etched surfaces performed more efficiently than sandblasted surfaces; furthermore, Sandblasted/etched surface results showed them to be more biocompatible, better tolerated, and appropriate for allowing hPDLSC growth and proliferation. The bone-titanium integration is rapid (20). Similarly, the double acid etched titanium surface is more biocompatible resulting in greater cell growth and adhesion, also increasing osteogenic and angiogenic processes compared to the machined titanium surface (21). 102

3. HUMAN PERIODONTAL LIGAMENT STEM CELLS (HPDLSCS) IN TITANIUM DENTAL IMPLANTS

The biological biomaterials are those that lead to natural tissue restoration. This goal is more easily achievable when the properties of a good biomaterial are combined with those of the stem cells abilities. Regenerative medicine is based on the use of stem cells, including adult MSCs, that can be used in cellular therapy to replace damaged cells or to regenerate tissues (22). MSCs are stromal cells characterized by: the ability to self-renew and multilinear differentiation. MSCs can be isolated from a variety of tissues, including the umbilical cord, bone marrow, adipose tissue, and also from menstrual blood and endometrium (23). Alternative sources of adult MSC have been identified that are readily available in tissues of the oral cavity (dental pulp, apical papilla, dental follicle, gingiva and periodontal ligament) (24, 25). In general, MSCs possess immunomodulatory properties since they are able to influence the immune response by interacting with components of the immune system and exhibiting antiinflammatory effects (26). In particular, human periodontal ligament stem cells (hPDLSC) have demonstrated not only the ability to differentiate into mesengen lineages, but also the ability to interact with immune cells (27), avoiding the improper activation of T lymphocytes. This demonstrates immunomodulatory properties of hPDLSC especially during the healing processes (28).

In several studies it has been demonstrated that biomaterials, including titanium, in association with hPDLSCs, determine a better yield of implant osteointegration thanks to the increase in osteogenic differentiation associated with a major release of matrix components such as fibronectin and collagen. Some titanium treatments in association with hPDLSCs culture show promising results. Li et al. have demonstrated that stimulation with Enamel matrix derivative promotes not only the proliferation of hPDLSCs cultured on the Ti surface but also their differentiation towards the osteogenic lineage, probably following the activation of the Protein kinase B/ mammalian target of rapamycin (Akt/mTOR) pathway (29). Chemically modified implants create a microenvironment that can improve osseointegration. A roughened and chemically modified implant surface increases the proliferation of hPDLSCs and increases osteoblastic differentiation. Furthermore, by regulating

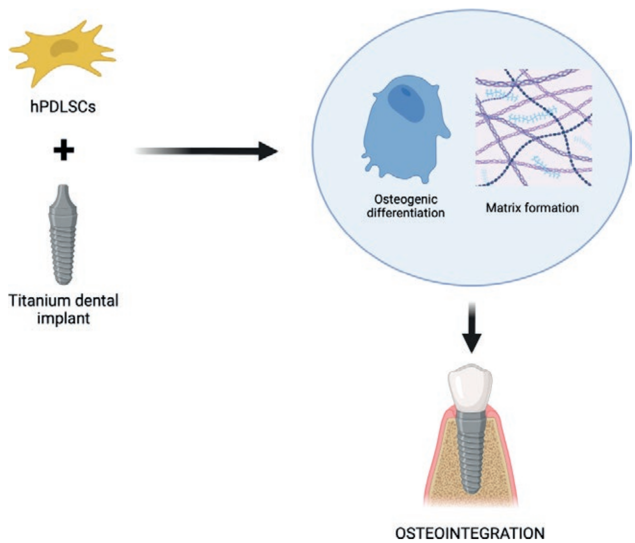


Figure 2. Interaction between titanium dental implant and human periodontal ligament stem cells (hPDLSCs) that leads to osteointegration trough osteogenic differentiation and matrix release. (Created with BioRender.com).

the RANKL-RANK-OPG axis, it interferes with osteoclastogenesis leading to its decrease (30). Titanium Carbide MXene promotes the osteogenic differentiation of hPDLSCs, probably through modulation of the Hypoxia-inducible factor 1-alpha/ wint (HIF-1α/WNT) signaling pathway related to metabolic reprogramming. This improves tissue regeneration and osseointegration (31).

4. VASCULARIZATION PROCESS IN TITANIUM DENTAL IMPLANTS

The term angiogenesis defines the process by which new capillary blood vessels are formed from existing ones. This process is governed by a balance of positive and negative angiogenic factors within the vascular microenvironment and is the result of the functional activity of these factors together with ECM proteins, adhesion receptors and proteolytic enzymes (32). Angiogenesis is a fundamental process during embryonic development and remains a physiological process even during adult life, in which it intervenes in processes such as the formation of the corpus luteum. On the other hand, angiogenesis is also the protagonist of pathological conditions, such as chronic inflammation and tumors, in which it can contribute to the progression of the disease (33). In vasculogenesis there is the differentiation of endothelial progenitor cells which are subsequently incorporated into the vessels (34). Angiogenesis is induced by various cytokines produced mainly by macrophages and platelets during the inflammatory phase, since the macrophage produces tumor necrosis factor alpha (TNFα) influencing the angiogenic process (35). Angiogenesis is a physiological process necessary for the growth and development of bodily structures in the human body as well as in tissue repair and regeneration (36). Vascular endothelial growth factor (VEGF), a member of the PDGF family of growth factors, is the key molecule of the vascularization process having a powerful angiogenic activity with a mitogenic and anti-apoptotic effect on endothelial cells. This increases vascular permeability as well as cell migration (37). Angiogenesis in regenerative dental practices is critical for the growth of new bone in bone regeneration of osseointegration after the installation of the dental implant, since a blood supply is required in order to provide nourishment, oxygen (38).

For the engraftment of titanium implants, the vascularization process is fundamental since the bone formation depends on it, which then closes the bone-implant interface. This process becomes even more important especially in the presence of large bone

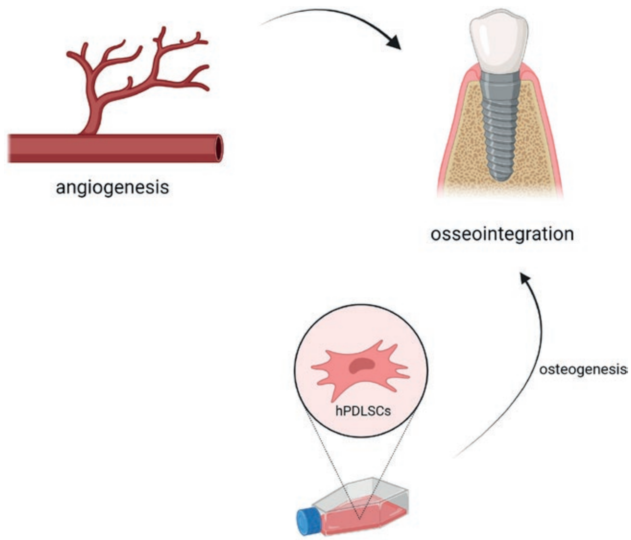


Figure 3. Vascularization in dental implant.

defects, in which early vascularization is a prerequisite, since a long distance must be bridged for the transport of nutrients, growth factors and for gas exchange (39). Increased levels of VEGF and its receptor could lead to faster bone-titanium integration. Furthermore, the expression of VEGF and consequently the vascularization process and therefore of osseointegration is increased in titanium implants with certain surface treatments compared to others. The hPDLSCs cultered on Sandblasted/etched surfaces have showed an higher VEGF expression respect to the cells seeded on sandblasted surfaces (20). Human PDLSC cultured on double acid etched titanium surface showed higher expression of VEGF compared to hPDLSC cultured on machined titanium surface (21).

5. DENTAL IMPLANTS: WOUND HEALING AND OSTEOINTEGRATION

The positioning of the dental implant gives rise to a series of biological reactions that allow bone remodeling. The first phase is bone turnover at the implant interface. The endpoint is represented by the integration of the implant with the absence of chronic inflammation and lack of mobility. This is followed by confirmation with radiographic evaluation of the reformed bone at the implant interface. Wound healing around the dental implant involves three stages of repair: initial formation of a blood clot, cellular activation, and finally by a cellular response (40). Adhesion to the implant surface leads to the activation of platelets, producing factors such as PDGF, TGF-Beta, PDEGF, IGF-1, induce the recruitment and differentiation of mesenchymal cells towards the bone tissue, accelerate the healing process (41). When titanium surfaces are modified with a controlled etching process, they alter platelet adhesion leading to the formation of thrombin-antithrombin complexes (42). In general, smoother machined surfaces demonstrated greater platelet adhesion but reduced activation; rougher surfaces determine reduced platelet adhesion but the relative degranulation is almost 100% (43). Platelets are followed by macrophages migration, which not only remove debris but also appear to mediate the formation of new bone on the implant surface and wound healing (44) (45, 46). The early macrophage population expresses growth factors such as fibroblast growth factor (FGF-1, FGF-2, FGF-4), transforming growth factors, epithelial growth factor, and bone morphogenetic proteins (BMPs) (47, 48). These factors influence processes such as cell recruitment, migration, proliferation and formation of an extracellular matrix on the implant surface, crucial for wound healing and angiogenesis since macrophages mediate the release of bFGF, TNF-α and VEGF (49). Another fundamental process for osseointegration is the formation of a mineralized matrix mediated by multipotent MSCs that differentiate into osteoblasts. The differentiation of osteoblasts is mediated by central binding factor alpha (Cbfa1) or RUNX-2, which is a transcription factor belonging to Runt that regulates the transcription of genes coding for bone sialoprotein (BSP), the osteocalcin and type I collagen (50, 51). Human MSCs cultured on titanium surfaces with a nanoetched topography show elevated expression of RUNX-2 and type I collagen with increased expression of alkaline phosphatase, required for biomineralization on the implant surface (52). Tissue regeneration and interaction with biomaterials is also mediated by the production of EVs, through which cells communicate and exchange functional material. EVs interact with matrix proteins such as fibronectin and type I collagen (53). Furthermore, EVs can induce and influence MSC differentiation in a certain direction, including the osteoblastic one in the process of osteogenesis (54). It has been demonstrated that the EVs produced by MSCs during the administration of dental implants, thanks to their immunomodulatory action, contribute to an increase in bone tissue density near the device and determine an almost complete fusion of the screw device with the bone tissue (55). Exosomes at the level of titanium disc implants significantly promote osteogenic differentiation and mineralization probably through microRNAs that influence mTOR, AMP-activated protein kinase (AMPK), Wnt pathways in the osteoimmune regulation mechanism of implant osseointegration (56). EVs derived from MSCs have been shown to contain microRNAs that can induce bone remodeling and mineralization such as miR-10b-5p, miR-21, miR-31-3p, miR31-5p, mRi-199a- 3p, miR-223-3p (57).

CONCLUSIONS

In summary, significant advances have concerned dental implant procedures and surface modification techniques for titanium dental implants. We provide a summary of titanium as a biomaterial in dental implants with related surface modifications, interaction with MSCs and EVs, in vascularization and wound healing processes on the base of osseointegration. However, further studies, especially in osseointegration from a molecular point of view, are necessary.

LIST OF ABBREVIATIONS

MSC: mesenchymal stem cell
Ti: titanium
ECM: extracellular matrix
EVs: extracellular vesicles
miRNA: micro RNA
hPDLSC: Human Periodontal Ligament Stem Cells
AKT/mTOR: activation of the Protein kinase B/ mammalian target of rapamycin
RANKL: Receptor activator of nuclear factor kappa-B ligand
RANK: Receptor activator of nuclear factor κ B
OPG: Osteoprotegerin
HIF-1α: Hypoxia-inducible factor 1-alpha
WNT: Wint
TNF-α: tumor necrosis factor alpha
VEGF: Vascular endothelial growth factor
PDGF: Platelet-derived growth factor
TGF-Beta: Tumor growth factor beta
IGF-1: Insuline-like growth factor
FGF-1; FGF-2; FGF-4: (fibroblast growth factor 1; 2; 4)
BMPs: bone morphogenetic proteins
bFGF: basic fibroblast growth factor
Cbfa1: central binding factor alpha
RUNX2: Runt-related transcription factor 2
BSP: bone sialoprotein
AMPK: AMP-activated protein kinase

AUTHOR CONTRIBUTIONS

Conceptualization: Y.D.R.,. and G.D.M.; writing—original draft preparation: Y.D.R. and G.D.M.; writing—

review and editing: G.D.M., Y.D.R., F.D., J.P., and A.M.; visualization: G.D.M., F.D., J.P., A.M, O.T. and Y.D.R.; supervision: G.D.M., J.P., and O.T. and A.P.; project administration: G.D.M., J.P., F.D., and O.T.

FUNDING

This work has been funded by the European Union - NextGenerationEU under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY – CUP: D73C22000840006.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Silva, G.B.C., Martini, C.S., Carvalho, R.P., Cunha, J.M., Centeno, R.S., & de Aguiar, P.H.P. (2024). White fibers anatomy through dissection - Klingler Method and its clinical correlation. *Italian Journal of Anatomy and Embryology* 128(2): 13-22. https://doi.org/10.36253/ijae-15400

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

White fibers anatomy through dissection - Klingler Method and its clinical correlation

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Abstract. White fiber anatomy is classified according to its function: association, commissural, and projection. The most studied are the superior longitudinal fascicle, inferior longitudinal fascicle, uncinate fascicle, and inferior frontal occipital fascicle, because of their anatomy and function. In this experimental investigative study in the laboratory, the Klingler technique was used for white matter fiber dissection of ten normal brains. During this period, we observed the anatomical and clinical correlation of the superior longitudinal fascicle, inferior longitudinal fascicle, uncinate fascicle, and inferior frontal occipital fascicle. This study allowed us to understand the important part of dissection in anatomy studies, even with the presence of more modern techniques such as tractography.

Keywords: superior longitudinal fascicle, white fibers, uncinate fascicle, inferior frontal occipital fascicle, inferior longitudinal fascicle.

INTRODUCTION

The white matter is a bundle of myelinated nerve fibers called fascicles or tracts. White matter fibers are classified according to their functions: association, commissural, and projection. The association fibers are of two types: the small ones, also called "U fibers," which connect adjacent gyri up to the lower portion of the sulcus, and the long ones, which connect brain lobes on the same side of the hemispheres, the most studied being the superior longitudinal fascicle (SLF), inferior longitudinal fascicle (ILF), uncinate fascicle (UF), inferior frontal occipital fascicle (IFOF) (Catani et al. 2002) . The commissural fibers interconnect the two cerebral hemispheres: corpus callosum, anterior commissure, and hippocampal commissure. On the other hand, projection fibers connect the cerebral cortex with the medulla and trunk, such as the corona radiata and internal capsule (Duffau 2011).

The SLF is divided into three parts. Its function is to connect the frontal lobe to the parietal and temporal ILF (Klingler and Gloor 1960, Janelle et al. 2022), with fibers connecting the temporal lobe to the occipital lobe (Janelle et al. 2022). UF, connects the frontal love to the temporal lobe, and IFOF, connects que frontal lobe to the occipital lobe (Klingler 1935).

One way to study white fibers is by dissecting brains, a method described by Josef Klingler in 1935 (Klingler 1935) that remains the most widely used method today. There are variations in the method, altered by the author himself in later works (Klingler and Gloor 1960), but it consists of three stages: fixation in formaldehyde, cooling, and thawing). A study by Dziedzic et al. (Dziedzic et al. 2021), who analyzed 37 articles regarding variations in Klingler's method, it was found that the time taken to fix formaldehyde varied from 8 h to 3 months, freezing from 8 h to 3 months, cooling temperature from -5 to 80°C, and formaldehyde solution for storage from to 4-10% in concentration. However, even with divergences, satisfactory results can be obtained, with the most important being the freeze-thaw process, as demonstrated by Zemmoura et al. (Zemmoura et al. 2016), because it preserves the myelin sheath; consequently, the structure of the axon, destroying the cellular matrix and glial cells, makes it easier to expose and separate the fibers during dissection.

This study specifically focused on dissecting three key white matter fibers: the uncinate fascicle, superior longitudinal fascicle, and inner frontal occipital fascicle. By dissecting these fibers, this study aimed to provide a deeper understanding of their anatomy and function.

MATERIALS AND METHODS

This was an experimental investigative study in the laboratory that meticulously utilized the Klingler technique to dissect white matter fibers (Guerrero et al. 2019). This technique, known for its precision, involves four distinct steps: fixation, freezing, unfreezing, and dissection (Klingler and Gloor 1960, Duffau 2011).

Ten normal adult brains were carefully fixed in 10% formalin solution for a minimum period of three months. Subsequently, the superficial veins and arachnoid membranes were meticulously removed. The brains were then frozen at temperatures between 0 °C and 5 °C for three weeks, a step that allows formalin to crystallize between the fibers, expanding and separating them for further preservation and observation. Subsequently, the samples were frozen and preserved in formalin. Dissection was performed using wooden spatulas (Klingler and Gloor 1960, Duffau 2011).

RESULTS

The dissection was made by completely removing the brain cortex (Figure 1), and in the lateral region of the encephalon, the U fibers were removed from the superior temporal gyrus, going up into the superior frontal gyrus, passing through the inferior frontal gyrus and medial frontal gyrus to reveal the partially horizontal and vertical superior longitudinal fasciculus. Subsequently, the U fibers were detached from the precentral, postcentral, superior parietal, angular, and supramarginal gyri to completely expose the horizontal superior longitudinal fasciculus.

The uncinate fasciculus was more internally localized than the superior longitudinal fasciculus. To locate it, removing the U fibers from the inferior and medial temporal gyrus, in addition to the short and long gyrus cortex of the insula, inside the Sylvian fissure is necessary. The inferior frontal occipital fasciculus is mainly located behind the frontal and occipital lobes and passes underneath the internal capsule.

Figure 1. Right hemisphere with remove córtex.

DISCUSSION

Superior longitudinal fasciculus (SLF)

Anatomy

The superior longitudinal fasciculus (SLF) - Figures 2-6 - is considered the largest associative fiber bundle system in the brain, which forms a wide arc around the upper edge of the insula and has the shape of a "C." The SLF is part of the longitudinal associative fiber system that forms connections between the frontal, parietal, occipital, and temporal lobes around the Sylvian fissure of the ipsilateral hemisphere (Türe et al. 2000, Jellison et al. 2004, Fernández-Miranda et al. 2008, Dini et al. 2013, Janelle et al. 2022).

The SLF was identified as the most lateral fasciculus with an anteroposterior orientation in color-coded diffusion tensor imaging (DTI) maps. On T2-weighted sagittal images, the SLF can be recognized as a white substance surrounding the posterior margin of the insula (Fernández-Miranda et al. 2008). It can be roughly divided into (I) longer fibers that run medially within the fasciculus and connect the lateral frontal cortex with the dorsolateral parietal and temporal cortex and (II) shorter U-shaped fibers that run more laterally and connect the frontoparietal, parieto-occipital, and parietal-temporal cortex (Catani et al. 2002). It can also be divided into three segments (one long, one anterior, and one posterior segment), each connecting two regions of Broca's, Wernicke's, or Geschwind's area (inferior parietal lobule) (Catani et al. 2002). The fibers originate in the prefrontal and premotor gyri (especially Broca's area) and project posteriorly to Wernicke's area (and the occipital lobe) before curving around the insula and putamen and running anteroinferiorly to the temporal pole. In addition, a few fibers originate in the insula of the Reil and project to the cortex of the other lobes (Catani et al. 2002).

At the beginning of the 19th century, Reil and Autenrieth identified the SLF based on postmortem brain dissection. The first description describes them as a group of fibers located in the white matter of the temporal, parietal, and frontal lobes. This description was further refined by Burdach, followed by Dejerine in 1895, who introduced a perisylvian arcuate fiber tract connecting the posterior temporal lobe to the frontal lobe. They called this fiber bundle the arcuate fasciculus (AF) and used the term 'superior longitudinal fasciculus' as a synonym. A century later, Petrides and Pandya studied rhesus monkeys using an autoradiographic technique and divided the SLF into three segments. These authors distinguished SLF and AF as two distinct entities with different orbits, thus blurring the classical description that prevailed at that time. The anterior segment of the AF appears to correspond to SLF III, and these two terms are used interchangeably. Thus, although these two bundles are distinct units, some of their subcomponents overlap, as if they share a subdivision: SLF III and the anterior segment of the AF (Janelle et al. 2022).

In a study by Schmahmann et al. (2007), the autoradiographic material (isotope) showed that the SLF I fiber bundle runs in the white matter of the superior parietal and frontal lobes. It lies dorsal to the CB and is distinct from it. It extends from the medial and dorsal parietal cortices to the dorsal part of the premotor and prefrontal cortices. SLF I connects the medial parietal areas PEc, PGm, 31, and the superior parietal lobe area PE with dorsal area 6, dorsal area 9, and the supplementary motor area (SMA) in the frontal lobe. Subcomponent II (SLF II) extends from the caudal inferior parietal lobe to the dorsal premotor and prefrontal cortices. It is located caudally in the white matter of the inferior parietal lobe and deep in the superior shoulder of the Sylvian fissure. Rostrally, it extends into the white matter below the premotor and prefrontal brain regions. SLF II connects area POa in the intraparietal sulcus (IPS) and areas PG and Opt of the inferior parietal lobe with areas BA 46, 9/46, 8Ad, and 6D of the frontal lobe. A subcomponent III fiber bundle (SLF III) was identified in the opercular white matter of the parietal and frontal lobes. It extends from the rostral inferior parietal lobe to the ventral part of the premotor and prefrontal cortices. The SLF III connects the area POa in the IPS and the areas PF, PFG, and PFop of the parietal lobe, as well as the ventral premotor area BA 6, 44, and the ventral prefrontal area 9/46v of the frontal lobe.

Figure 2. Horizontal superior longitudinal fasciculus. H-SLF: Horizontal Superior Longitudinal Fasciculus, EC: external capsule.

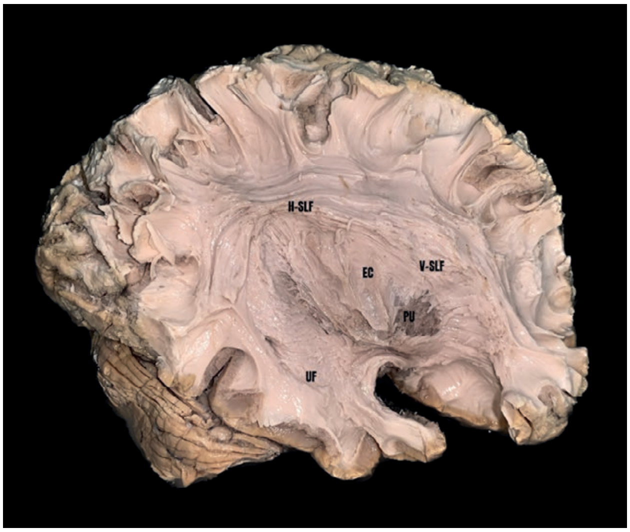

Figure 3. Superior longitudinal fasciculus and uncinate fasciculus. H-SLF: Horizontal Superior Longitudinal Fasciculus, V-SLF: Vertical Superior Longitudinal Fasciculus, EC: external capsule, PU: Putamen, UF: Uncinate Fasciculus.

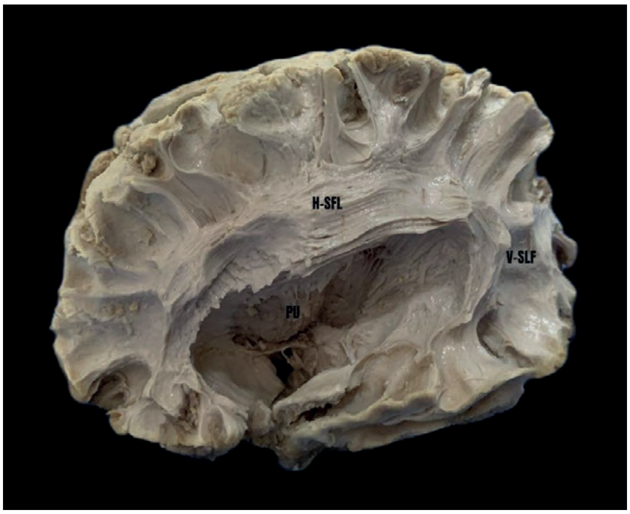

Figure 4. Horizontal and Vertical superior longitudinal fasciculus. H-SLF: Horizontal Superior Longitudinal Fasciculus, V-SLF: Vertical Superior Longitudinal Fasciculus, PU: Putamen.

Anatomical variability can be observed at different levels in cortical morphology, cytoarchitecture, task-related activation, or dMRI connectivity patterns. Specifically, there are within-population differences in the SLF; the volume of the SLF may differ from individual to individual. The fractional anisotropy of the SLF also varies between individuals but is less than the volume (Janelle et al. 2022).

Figure 5. Horizontal superior longitudinal fasciculus. H-SLF: Horizontal Superior Longitudinal Fasciculus.

Figure 6. Horizontal and Vertical Superior longitudinal fasciculus. H-SLF: Horizontal Superior Longitudinal Fasciculus, V-SLF: Vertical Superior Longitudinal Fasciculus.

Dissecting description

Martino et al.(Martino et al. 2013) modified the classical method for dissecting fibers originally designed by Klinger. The aim was to preserve the cortex by removing only a minimum amount of tissue during dissection, thus enabling cortex-sparing dissection of the fibers. This approach identified two superficial segments of the SLF: the first, horizontally oriented, connects the inferior parietal lobe and the posterior part of the superior temporal gyrus to the frontal operculum. The second component runs along the AF and connects the posterior part of the middle temporal gyrus to the posterior part of the inferior parietal lobe (the angular gyrus). A deeper fiber segment corresponding to classic AF has also been identified (Janelle et al. 2022).

The cortical gray matter and the adjacent superficial short fibers of the frontal, temporal, and parietal operculum; the middle frontal, superior, and middle temporal gyri; and the inferior parietal lobe must be removed to expose the SLF. The removal of the short fibers exposed the deeper long association fibers that descended from the gyri and traveled a variable distance to the distant gyri. The horizontal orientation of the long fibers at the depth of the inferior and middle frontal gyri, where they form a compact fasciculus of approximately 20 mm lateromedial diameter located 22–25 mm from the cortical surface, is evident (Fernández-Miranda et al. 2008). At the level of the temporoparietal junction area and approximately 20 to 25 mm from the cortical surface, a well-defined group of vertically oriented fibers running between the posterior part of the middle and superior temporal gyri and the region of the inferior parietal lobe can be seen (Fernández-Miranda et al. 2008). They form temporoparietal or vertical segments of the SLF. At a deeper level in the temporoparietal area, a group of fibers curving around the posterosuperior insular border and running between the posterior temporal and prefrontal regions can be seen. They form the frontotemporal or arcuate segments of the SLF (Fernández-Miranda et al. 2008).

Clinical correlation

Each segment of the dominant superior longitudinal association system can be associated with a specific disorder: the frontoparietal segment with non-fluent aphasia, temporoparietal segment with intelligible aphasia, and frontotemporal or arcuate segments with conduction aphasia. Frequent combinations of white matter lesions can cause aphasic disorders (Fernández-Miranda et al. 2008).

The frontoparietal segment of the SLF connects the prefrontal region with the inferior parietal lobule. The latter is a high-order association cortex that integrates inputs from different modalities and plays a vital role in spatial function in the nondominant hemisphere. The nondominant prefrontal region plays a vital role in regulating visual attention in different parts of the space. Thus, the non-dominant frontoparietal segment may serve as a conduit for visuospatial perception (Fernández-Miranda et al. 2008).

The temporoparietal segment of the SLF connects the inferior parietal lobule with the superior temporal gyrus, which is associated with auditory information processing. Thus, the non-dominant temporoparietal (indirect pathways) and arcuate (frontotemporal and direct pathways) segments of the longitudinal superior fasciculus can process audio spatial and visuospatial information, respectively (Fernández-Miranda et al. 2008).

SLF III has similar connectivity to the AF and connects the inferior frontal gyrus to the ventral precentral gyrus. This suggests that it plays a role in speech in the left hemisphere, which was confirmed by Maldonado et al. through a brain electrical stimulation study. Wang et al. also reported a specific connectivity pattern for the suitable SLF III, which terminates at the proper pars triangularis. They found a solid leftward orientation of connections between the supramarginal gyrus, dorsal precentral gyrus, and caudal middle frontal gyrus. This fits with the role of the left SLF II in motor planning of speech and syntax processing. In a similar situation to SLF III, a suitable SLF II preferentially connects the angular gyrus and the superior parietal lobe with the caudal or rostral middle frontal gyrus. It is assumed that this system plays a role in regulating attention in spatial orientation. This is consistent with SLF II being responsible for faster and preferential visuospatial processing in the right hemisphere (Maldonado et al. 2012, Wang et al. 2016, Janelle et al. 2022).

Uncinate Fasciculus (UF)

Anatomy

The uncinate fasciculus (UF) - Figure 7-9 - is an essential long-range white matter association fiber tract that connects the anterior temporal lobe to the medial and lateral orbitofrontal cortices via a direct, bidirectional monosynaptic pathway (Türe et al. 2000, Catani et al. In 2002, Schmahmann, et al. 2007, Fernández-Miranda et al. 2008, Papagno et al. 2011, Von Der Heide et al. 2013). The UF is the most rostral fiber bundle in the temporal lobe (Schmahmann et al. 2007). It runs caudally through the white matter of the frontal lobe, bends sharply ventrally in the region of the limen insulae, and then spreads to reach the cortex of the anterior part of the superior and middle temporal gyri (Türe et al. 2000). UF has a pronounced hook shape that runs in an arc around the Sylvian fissure (Schmahmann et al. 2007, Von Der Heide et al. 2013). “Uncinate,” from the Latin uncus, means “hook-shaped.” It curves around the lateral sulcus and connects the inferior and orbital frontal gyri to the anterior temporal lobe (Dini et al. 2013).

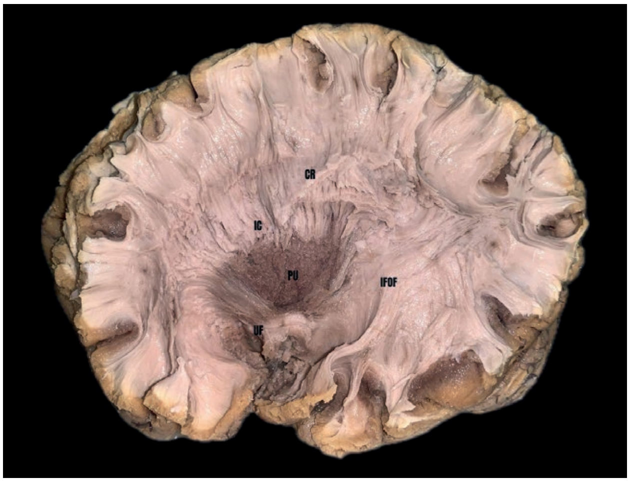

Figure 7. Uncinate Fasciculus and Inferior Fronto-occipital Fasciculus. UF: Uncinate Fasciculus and Inferior Fronto-occipital Fasciculus, IFOF: Inferior Fronto-occipital Fasciculus, CR: Corona Radiata, IC: Internal capsule, PU: Putamen.

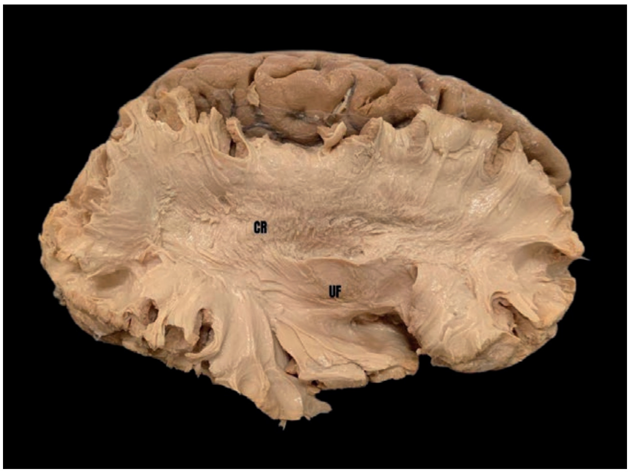

Figure 8. Uncinate Fasciculus. UF: Uncinate Fasciculus and Inferior Fronto-occipital Fasciculus, CR: Corona Radiata.

The UF is often divided into three parts: dorsal/temporal segment, middle/insular segment, and ventral/frontal extension. The three main areas of the rostral temporal lobe are connected to the frontal lobe: the TS1 area of the rostral superior temporal region and the temporal pro-iso-cortex; the inferior temporal areas TEa, IPa, and TE1; and the ventromedial temporal areas TH, TL, and TF in the parahippocampal gyrus, entorhinal cortex, and amygdala (Schmahmann et al. 2007) . The temporal segment originates from the uncus (Brodmann area [BA] 35), entorhinal and perirhinal cortex (BA 28, 34, and 36), and temporal pole/anterior temporal lobe (BA 20 and 38). BA 10, 11, 47/12, 13, 14, and 25 were connected to the orbital areas of the frontal lobe. The lateral prefrontal cortex originates or terminates in areas 10 and 47/12, and on the medial surface in areas 32 and the rostral area 24 (Schmahmann et al. 2007, Von Der Heide et al. 2013).

It is more prominent in the right hemisphere, indicating a more robust frontotemporal connectivity. The temporal fibers lie medial and anterior to the fibers of the inferior longitudinal fasciculus (Papagno et al. 2011). The anterior part of this tract is located inferior and medial to the fronto-occipital fasciculus. The UF borders the fronto-occipital fasciculus in its middle part before curving inferiorly and laterally towards the temporal pole and middle and superior temporal gyri (Dini et al. 2013). The UF then enters the outer capsule, and its fibers project medially to the insula and laterally to the lenticular nucleus (Dini et al. 2013).

In tractography, the dorsal and more lateral fibers run posteriorly from the frontal pole and unite with a more ventral and medial branch from the orbital cortex to form the uncinate. This runs a short distance below the frontal-occipital fasciculus before entering the temporal lobe as a single compact bundle in which the two divisions can still be distinguished. The uncinate fasciculus then forms an anteromedial hook end in the temporal pole, uncus, hippocampal gyrus, and amygdala (Catani et al. 2002).

The cell bodies of the UF are located in the temporal segment. From there, the UF runs upward across the lateral nucleus of the amygdala, through the limen insulae, and either near or through two smaller white matter tracts, the external capsule and the extreme capsule (Catani et al. 2002, Von Der Heide et al. 2013). In this region, the UF is inferior to the fasciculus frontalis occipitalis. Subsequently, it merges into the orbital regions of the frontal lobe (BA 11 and 47), where it has a horizontally oriented fan shape. The fan is divided into two branches: giant ventrolateral and smaller medial branches. The ventral branch ends in the lateral orbitofrontal cortex, whereas the medial branch ends in the frontal pole (BA 10). In adult humans, the UF has a width of 3–7 mm, height of 2–5 mm, and volume of ~140 mm3 (Schmahmann et al. 2007, Von Der Heide et al. 2013).

Dissecting description

The UF was exposed on the cortical surface of the limen insulae. This thick, hook-shaped fasciculus forms the anterior part of the frontotemporal junction (also called the temporal stem) and, in its lateral part, connects the frontal-orbital region with the temporal pole to form part of the ventral part of the extreme and external capsule. When the UF fibers were removed, several island-like grey matter masses interspersed with the fibers were exposed. These islands of gray matter form the ventral claustrum, which is connected in the superficial plane to the dorsal claustrum located above and behind the UF. The UF was dissected medially. In this case, the white fibers of the ventral part of the external capsule are exposed, connecting the frontonasal (gyrus rectus, subcallosal area) and temporomesial areas, as well as the gray matter of the ventral claustrum, which merges into the nucleus amygdaloid, located anteromedially of the UF (Wang et al. 2016).

Clinical correlation

UF has been associated with various developmental and psychiatric disorders. Its location makes it susceptible to direct impact and shear injuries from head trauma. It is frequently associated with white matter damage and traumatic brain injuries. The anterotemporal lobe is typically associated with the limbic system and its functions are thought to be related to emotion and episodic memory (Von Der Heide et al. 2013).

The intertemporal lobe is involved in processing modality-specific information, such as auditory (rostral supratemporal gyrus), visual (rostral inferotemporal region), somatosensory and gustatory (rostral insular operant cortex), mnemonic (parahippocampal gyrus), and emotional (amygdala) information. The orbitofrontal area is involved in the regulation of behavior, emotions, decision-making, and self-regulation. The UF acts as a link between emotion and cognition (Fernández-Miranda et al. 2008, Von Der Heide et al. 2013).

Several studies have reported altered functional activity in the frontal regions, along with increased activation in the limbic/paralimbic regions, in generalized anxiety disorder and social anxiety disorder. Some studies have reported that the neuronal regions connected by the UF (orbital frontal cortex and temporal pole) are relatively thinner or have a lower volume in psychopaths and individuals with antisocial personality disorder than in controls (Von Der Heide et al. 2013).

The literature discusses three functions of the UF: associative and episodic memory, linguistic, and social-emotional functions. Episodic memory-reversal learning; learning from feedback/rewards/punishments; forming associations that motivate behavior; value-based updating of stored representations. Language retrieval of proper names for individuals, possibly some aspects of semantic memory retrieval. Social-emotional processing: appraisal of stimuli, processing of social rewards, overriding the emotional meaning of concepts.

A literature review (Von Der Heide et al. 2013) found no evidence to support the claim that the UF plays a primary role in anxiety disorders or schizophrenia but found more substantial evidence for UF dysfunction in psychopathy and epilepsy.

The uncus is part of the olfactory cortex, the entorhinal cortex is closely associated with hippocampal episodic memory functions, the perirhinal cortex has a controversial function in object perception and high-level object memory, and the temporal pole and adjacent tissue form part of the anterior temporal lobe. Parts of the anterior temporal lobe (but not the temporal pole) are thought to play a role in certain types of semantic memory, and in the encoding and storage of social and emotional concepts (Von Der Heide et al. 2013).

Interruption of the UF during anteromedial temporal lobectomy or transsylvian transinsular selective amygdalohippocampectomy for medial intractable temporal lobe epilepsy may be associated with the psychosocial clinical improvement observed after surgery, perhaps because the UF can no longer transmit pathological information from the temporal lobe to decision-relevant regions of the orbitofrontal cortex (Fernández-Miranda et al. 2008).

The UF plays a role in recalling personal names, as its removal results in a significant deficit, even when the temporal pole is spared. Furthermore, the results support the hypothesis that the retrieval of conceptual knowledge is separate from the retrieval of names (Papagno et al. 2011).

Inferior fronto-occipital fasciculus (IFOF)

Anatomy

The Inferior Fronto-Occipital Fasciculus (IFOF) (Figures 7 and 9) represents one of the most extensive white matter tracts bridging the occipital lobe to the anterior regions, including the temporal and frontal lobes (Oishi et al. 2011). Understanding the inferior frontal-occipital fasciculus is essential to elucidate its cognitive and perceptual processing functions. Neuroscientific studies have used advanced brain imaging techniques such as magnetic resonance imaging and diffusion tractography to map the trajectory and connectivity of these nerve fibers. This knowledge is essential to better understand the role of the inferior frontal-occipital fasciculus in neurological and psychiatric conditions as well as to develop more effective diagnostic and treatment strategies for disorders that affect brain function (Surbeck et al. 2020).

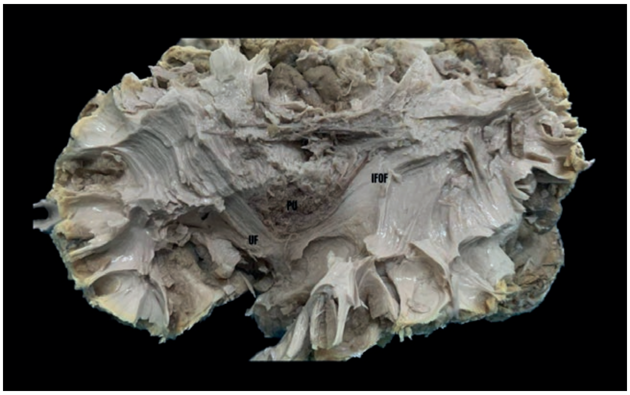

Figure 9. Uncinate Fasciculus and Inferior Fronto-occipital Fasciculus. Uncinate Fasciculus and Inferior Fronto-occipital Fasciculus, IFOF: Inferior Fronto-occipital Fasciculus, PU: Putamen.

The IFOF spans across the occipital, parietal, and temporal cortices within the sagittal stratum, converges with the roof of the temporal horn, extends to the ventral aspect of the external/extreme capsule, proceeds beneath the insular lobe within the temporal stem, and ultimately terminates within the frontal lobe (Martino et al. 2013). Situated internally from the Uncinate Fasciculus (UF) (Wu et al., 2022), the IFOF exhibits a notable division into superficial and deep components at the ventral aspect of the external capsule. The IFOF superficial portion arches mediolaterally at the superior level, limiting the insular sulcus before culminating in the Inferior Frontal Gyrus (IFG) (Wu et al. 2022).

It is important to know that the IFOF can be classified into two parts: (I) a superficial dorsal component, which establishes connections between the pars triangularis and orbitalis with the superior parietal lobe and the posterior segments of the superior and middle occipital gyri, and (II) a deep ventral component, which establishes connections between the posterior segment of the inferior occipital gyrus and the posterior basal temporal region with three distinct areas within the middle frontal gyrus (MFG), dorsolateral prefrontal cortex (DLPFC), and orbitofrontal cortex (Oishi et al. 2011).

Dissecting description

The superficial and dorsal subcomponent fibers of the IFOF connect to different cortical regions of the parietal and occipital lobes. In the superior parietal lobe, the IFOF dorsal fibers, which course through the superior portion of the sagittal stratum along the superior aspect of the atrium lateral surface, determine their trajectory at the superior parietal lobe convexity surface. In addition, the superior and middle occipital gyri fibers run through the middle portion of the sagittal stratum along the superior part of the occipital horn lateral surface, terminating in the posterior regions of the superior and middle occipital gyri (Martino et al. 2013).

Moreover, the deeper and ventral subcomponents of the IFOF are associated with the regions of the occipital and temporal lobes. The fibers directed towards the inferior occipital gyrus navigated through the inferior portion of the sagittal stratum and along the inferior aspect of the lateral surface of the occipital horn, concluding their trajectory in the posterior part of the inferior occipital gyrus. Meanwhile, fibers directed towards the posterior and basal temporal regions diverge from the main bundle to encircle the inferior border of the atrium and occipital horn, eventually terminating in the posterior part of the fusiform gyrus, temporo-occipital sulcus, and inferior temporal gyrus basal surface (Martino et al. 2013).

Clinical correlation

Studies utilizing electrostimulation of white matter pathways have revealed that the IFOF holds significant relevance in semantic processing, as it serves as a connecting channel between two areas associated with this function: the occipital associative extrastriate cortex and the temporal-basal region. In this context, intraoperative electrical stimulation (IES) of the IFOF induces semantic paraphasia, which means that the action can be executed. However, this did not correspond to the developer's intent (Duffau et al. 2008). Recent research has highlighted that the IFOF from the left hemisphere is related to semantic working memory (Horne et al. 2022) and that the right IFOF has been associated with emotion recognition (Philippi et al. 2009).

CONCLUSION

In this study, we applied the Klinger Method to dissect SLF, UF, and IFOF. By delineating their steam anatomically, we can understand the anatomy and function of the fasciculus more clearly. In MRI fiber tractography, there is considerable variability in segmentation protocols, fiber overlap, and techniques that affect the results, which the dissection would not deal with, as it enables the differentiation of one fiber from another and allows a better understanding of white fiber anatomy and function.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Melovitz-Vasan, C., Huff, S., & Vasan, N. (2024). Complex and multiple anomalies of the aortic arch: Atypical origin of the vertebral artery of continuing interest among embryologists, anatomists, and clinicians. *Italian Journal of Anatomy and Embryology* 128(2): 23-29. https://doi.org/10.36253/ijae-15440

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Complex and multiple anomalies of the aortic arch: Atypical origin of the vertebral artery of continuing interest among embryologists, anatomists, and clinicians[1]

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Abstract. Aortic arch branching anomalies are a relatively rare occurrence, observed in approximately 3-5% of cadavers. These anomalous branching patterns continue to be a topic of ongoing dialogue in embryological evolution and cervical region surgery. During dissection, it was discovered that the donor's left vertebral artery arose from the aortic arch between the left common carotid and subclavian arteries. In an earlier report that is included here, the right vertebral artery arose directly from the aortic arch (authors and journal expressed permission and attribution: Vasan et al., 2022). This paper not only discusses the embryological evolution of the vertebral artery and its anomalous branching but also emphasizes the need for careful examination of the aortic arch and its branches during clinical evaluation and surgical procedures.

Keywords: anomalous origin of vertebral arteries, altered preforaminal vertebral arteries, difference in vertebral artery length.

INTRODUCTION

The introduction includes a historical perspective that starts in the early 20th century and focuses on the number of primary branches of the aortic arch. The normal arch of the aorta has three branches: the right brachiocephalic trunk, also known as the innominate artery, which bifurcates into the right subclavian and right common carotid arteries, while the left common

[1] Disclosure: Part of this case report was presented as a poster at the Experimental Biology 2022 meeting hosted by the American Association for Anatomy on April 4th, 2022, in Philadelphia, PA, USA.

carotid and left subclavian artery follow the brachiocephalic trunk. This ‘typical’ pattern of aortic arch branches occurs in about 70% of the population (Rosen, 2023). To understand the multiple anomalies of the aortic arch, it is important to briefly discuss its embryological origin.

The development of the aorta and its branches in humans occurs during the third week of gestation (Schleich, 2002; Moore et al., 2020) and is a highly regulated and complex process that involves several synchronized events occurring sequentially or concurrently. Additionally, several intrinsic and extrinsic factors/molecules appear transiently with specific functions in normal development before disappearing (Moore et al., 2020). Due to the complexity of the development of the aorta and aortic arches, several congenital abnormalities are possible.

Some reports focused on the number of primary branches of the aortic arch (Poynter, 1916; Congdon, 1922; Iyer, 1927; Adachi, 1928; Windle et al., 1928; Lippert & Pabst, 1985). In some cases, however, the number of primary branches may be increased through other brachiocephalic arteries such as the thyroidea ima, internal and external carotid, right vertebral, internal thoracic, and inferior thyroid arteries. Infrequently, the right subclavian artery has been found to originate directly from the aortic arch as the last branch and to follow a retroesophageal course similar to the retroesophageal right subclavian artery (RRSA) (Haughton, 1974).

During weeks four to eight of embryonic development, the vertebral artery starts to form and the horizontal part of the 1-6 intersegmental arteries (ISA) begins to recede. By the development of longitudinal anastomoses that link the cervical ISA, the seventh ISA becomes the proximal subclavian artery. The adult vertebral artery usually originates from the subclavian artery and develops as a longitudinal channel connecting the cranial intersegmental arteries. If the left seventh intersegmental artery is incorporated into the developing aorta, it results in the left vertebral artery arising directly from the aortic arch. If one of the first six ISAs fails to involute (known as the persistent ISA), it can cause abnormal origins of the vertebral artery (Bordes et al., 2021; Dumitrescu et al., 2021).

Occasionally, the left vertebral artery arises directly from the aorta between the left common carotid and left subclavian arteries. This occurs when one of the proximal six intersegmental arteries (ISAs) fails to involute, resulting in abnormal origins of the vertebral artery (Lazaridis et al., 2018; Natsis et al., 2021). A persistent ISA located in the upper ISAs leads to an abnormal origin of the vertebral artery from the internal or external carotid artery (Lazaridis et al., 2018; Natsis et al., 2021). On the other hand, if it occurs in the lower 3-6 ISAs, it results in an abnormal origin of the vertebral artery from the aortic arch or the common carotid artery (Haughton, 1974.). In around 3-5% of individuals, the left vertebral artery arises directly from the aortic arch, which is clinically relevant during cervical region surgery (Matula et al., 1997).

The vertebral artery is separated into four segments based on its anatomical location. The first segment, the pre-foraminal or proximal segment, runs from its origin to the transverse foramen of C5/C6. The second segment, known as the intraforaminal segment, passes through the transverse foramen of the cervical vertebra. The third segment is the extradural segment; it runs from the second cervical transverse foramina to the base of the skull. The fourth and final segment, the intracranial segment, joins the vertebral artery from the other side to form the basilar artery (Matula et al., 1997). Bilaterally, the vertebral arteries show an expansion proximally at their origin and just before they enter the vertebral foramen (Vasan, 2022).

MATERIALS AND METHODS

The cadaveric specimens in the present study were obtained from the willed body program for medical student dissection. During routine cadaveric dissections of the thorax by medical students, we observed that both the right and left vertebral arteries atypically originated from the aortic arch (AA). The right vertebral artery originated from the right brachiocephalic trunk (Figs. 1, 3, and 4), while the left vertebral artery originated from the aortic arch between the left common carotid and the left subclavian arteries.

Donor cause of death

The donors were a 94-year-old Caucasian female who died of right femur fracture sequelae (Figs. 1, 2) (Case 1); a 73-year-old Caucasian female who died of renal failure following a complicated urinary tract infection (Fig. 3) (Case 2), and an 82-year-old Caucasian male with coronary artery disease and severe aortic stenosis (Fig. 4) (Case 3). Cases 2 and 3 and their related figures appeared in the authors’ prior publication and, as such, are allowed to be used by including appropriate attribution for the work given.

Declaration

The authors state that every effort was made to comply with all the local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research.

RESULTS AND OBSERVATION

As beginners, the students did not perform a good-quality dissection in Case 1. However, upon our own further careful dissection, we observed that the right vertebral artery emerged from the brachiocephalic trunk (Fig. 1). The left vertebral artery emerged from the aortic arch between the left common carotid artery and the left subclavian artery (Fig. 2). Proximally at its origin, both the right and left vertebral artery exhibited recognizable distension, which was also noticed before entering the foramen transversarium (Figs. 1,2).

Figures 1 and 2 show clear differences in the length of the preforaminal section of the right (5.5 cm) and left (8.0 cm) vertebral arteries. It is important to note that the right and left vertebral arteries enter the foramen transversarium at the 5th and 6th cervical vertebrae, respectively. Additionally, both right and left vertebral arteries exhibit distension before entering the foramen transversarium.

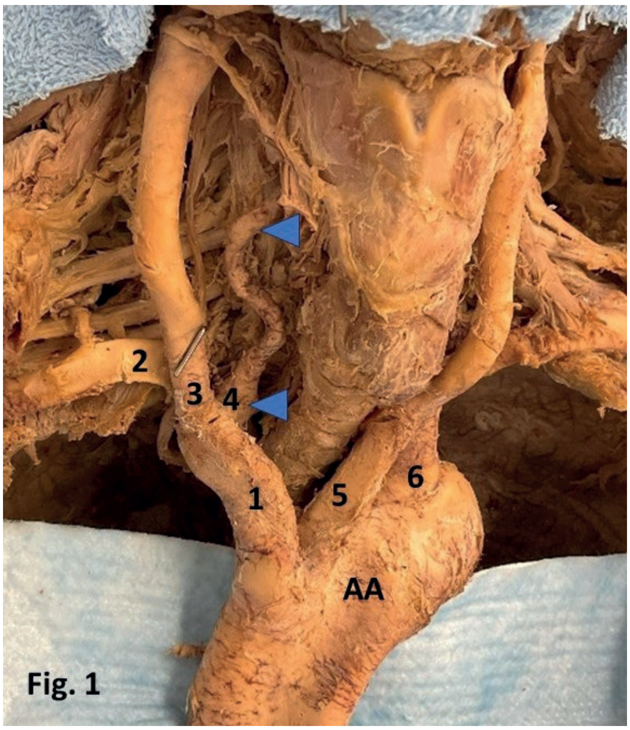


Figure 1. AA: Aortic Arch; 1: Right brachiocephalic trunk. 2: Right Subclavian Artery; 3: Right Common Carotid Artery; 4: Right Vertebral Artery; 5: Left Common Carotid Artery 6: Left Subclavian Artery. Blue arrowheads show the dilatations in the vertebral artery.

Noticeable differences in the preforaminal length of the vertebral arteries are significant. As seen in Cases 2 and 3 (Figs. 3, 4), the right vertebral artery measures 5.5 cm, while the left vertebral artery measures 7.0 cm. Additionally, the right and left vertebral arteries entered the foramen transversarium at the 5th and 6th cervical vertebrae, respectively. There was no difference either in the diameter or lumen size between the right and left distal parts of the vertebral arteries. Both right and left vertebral arteries showed a 'kink' and distension prior to entering the foramen transversarium.

DISCUSSION

The brain's blood supply comes from the left and right internal carotid arteries, known as anterior circulation, which comprises most of the cerebral hemispheres,

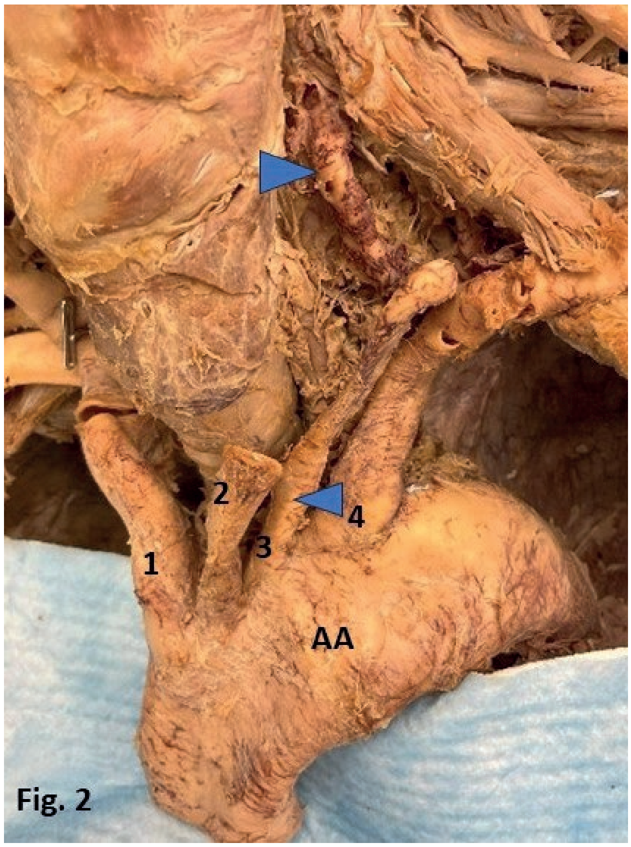


Figure 2. The Right (see Fig. 1) and Left Vertebral Arteries entered the foramen transversarium at C5 and C6, respectively. Before entering the foramen, the vertebral arteries exhibited dilatation, as indicated by the blue arrowheads. 1: Right brachiocephalic trunk. 2: Left Common Carotid Artery (trimmed to expose the Left Vertebral Artery. 3. Left Vertebral Artery. 4: Left Subclavian Artery.

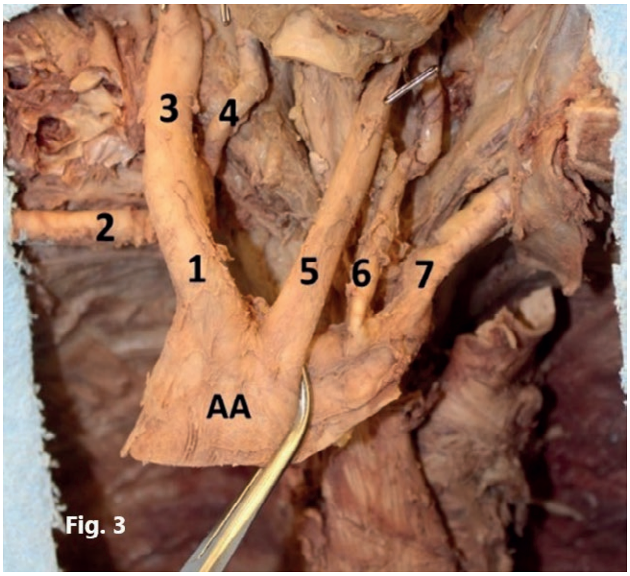
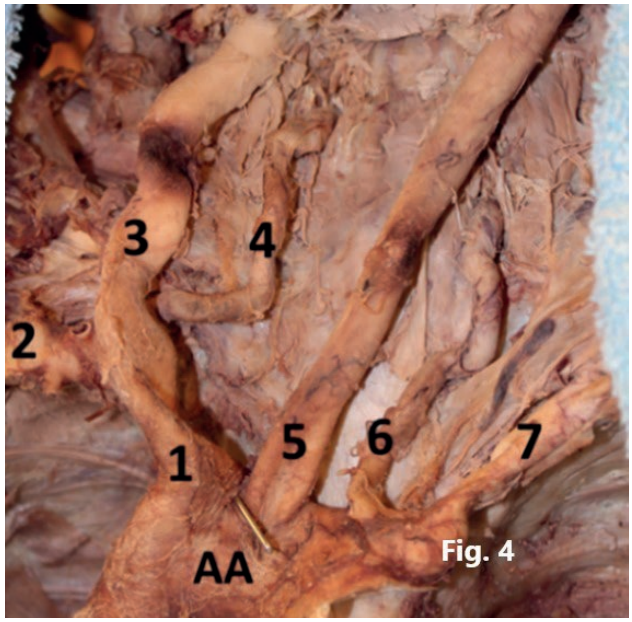


Figure 3. AA: Aortic Arch; 1: Right brachiocephalic trunk 2: Right Subclavian Artery 3. Right Common Carotid Artery 4: Right Vertebral Artery 5: Left Common Carotid Artery 6: Left Vertebral Artery, 7: Left Subclavian Artery.



Figures 3 and 4: Both the Right and Left Vertebral Arteries entered the foramen transversarium at C6 and C5, respectively. Prior to entering the foramen, the vertebral arteries exhibited dilatation. Figures 3 and 4 are from our earlier publication (approved by the journal policy): Int J Anat Res 2022, Vol 10(3):8426-8429. Https://doi.org/https://doi.org/10.16965/ijar.2022.17.

including frontal lobes, lateral temporal lobes, and the anterior part of deep cerebral hemispheres. The vertebral arteries are part of the posterior circulation of the brain that supplies the brainstem, cerebellum, occipital lobes, medial temporal lobes, and the back part of the deep cerebral hemisphere, specifically the thalamus. Regardless of their origin, the vertebral arteries contribute to the posterior brain circulation. The Circle of Willis connects the anterior and posterior circulations through anastomosis.

Individuals born with a left vertebral artery originating from the aortic arch may not experience any symptoms. However, there is a risk of the abnormal origin of the right vertebral artery becoming dilated, which can lead to cerebrovascular disorders and atherosclerotic changes due to increased blood flow (Tardieu et al., 2017). It is crucial to identify the anomalous origin and anatomical positioning of the left vertebral artery when considering anterior cervical spine surgery or other head-neck procedures that require retracting soft tissue structures such as nerves, arteries, veins, and muscles to reach the cervical spine (Tardieu et al., 2017). The prevertebral part of the vertebral artery, particularly the extracranial portion, is frequently affected by atherosclerosis and is a common site of stenosis (Imre et al., 2010). In Case 1, a sclerotic, narrower left vertebral artery was discovered, while the right vertebral artery was dilated due to increased blood flow, which helped protect the brain from ischemia (Melovitz-Vasan et al., 2015; Vasan et al., 2022).

Matula noted that the prevertebral vertebral artery tends to have a winding path in about 39% of cases, which is consistent with our observation (Figs. 1 and 2) and an earlier report (Matula et al., 1997; Freilich et al., 1986). Additionally, there is a difference in the twistiness of the right and left vertebral arteries, with 32% of right vertebral arteries being twisted versus 68% of left vertebral arteries. While the twists in the distal or preforaminal segment of the vertebral artery do not have any hemodynamic consequences, the loops of the proximal segments have been known to cause nerve root compressions, leading to radicular symptoms (Matula et al., 1997).

Previous studies confirmed that individuals with Down syndrome have a greater likelihood of having vascular anomalies; they also report a 40% occurrence rate of vertebral artery anomalies and a 36% occurrence rate of aberrant Right Subclavian Carotid Artery (RSCA) in these individuals (Rathore, 1989; Mishra et al., 2012). The deletion of Chromosome 22q11, also known as CATCH 22, is commonly associated with DiGeorge syndrome, conotruncal anomaly face syndrome, and velocardiofacial syndrome. Patients with this deletion are more likely to have anomalies of the aortic arch, aortic branches, ductus arteriosus, and pulmonary arteries than those without the deletion (Momma et al., 1999).

ANALYSIS

Anomalous origin of the right vertebral artery is less common than that of the left vertebral artery. The plausible embryologic explanation can only be speculative. Although these findings may be incidental in most cases, detailed information is required before any surgical/endovascular intervention to avoid any misinterpretation and inadvertent injury to the vertebral artery.

The following analysis pertains to the cases presented here and is based on our observations, findings, and published research studies on vertebral artery origin, malformations, and their effect on anatomy and physiology. In the analysis, we considered the age and gender of the donors and the cause of death. Any conclusion drawn in the analysis is the authors' or is based on available published materials. During routine cadaveric dissection of the thorax in Case 1, we observed that both the right and left vertebral arteries originated atypically. The right vertebral artery originated from the brachiocephalic trunk (Fig. 1), while the left vertebral artery originated between the left common carotid and the left subclavian arteries (Fig. 2).

In general, vertebral artery anomalies result from their development during embryonic stages, their path through the cervical region of the neck, and the morphology of the four individual segments. The vertebral artery begins to form during weeks four to eight of embryonic development, during which time the horizontal part of the 1-6 intersegmental arteries (ISA) starts to regress. The seventh ISA becomes the proximal subclavian artery by developing longitudinal anastomoses that link the cervical ISA. If the left seventh intersegmental artery is incorporated into the developing aorta, it results in the left vertebral artery, often arising directly from the aorta between the left common carotid and left subclavian arteries (Bordes et al., 2021).

Normally, the right vertebral artery arises as a branch from the right subclavian artery. However, it is possible that, while the right fourth arch is incorporated into the right subclavian artery, the dorsal aortic roots between the third and fourth arches persist and become the right vertebral artery.

Regardless of their origin, the vertebral arteries are significant because they form part of the brain's posterior circulation, and the two internal carotid arteries provide the anterior circulation. The Circle of Willis connects the anterior and posterior circulation.

Our present study revealed that the diameter, length, and tortuosity of the prevertebral segment of the left and right vertebral arteries varied (Figs 1-4). Additionally, based on the available literature, these observed variations in physical nature likely contributed to blood flow and hemodynamics. A significantly enlarged foramen transversarium was discovered incidentally during a cone-beam computed tomography scan. Upon further evaluation with magnetic resonance angiography, Omami (2021) discovered that the foramen enlargement was caused by a tortuosity in the vertebral artery's course.

Our report includes what was observed in the cases presented here, but our findings mirror earlier discoveries (Poynter, 1916; Congdon, 1922; Iyer, 1927; Adachi, 1928). Irrespective of when and who conducted the studies, the genesis of the vertebral artery occupied the interests of embryologists, anatomists, and clinicians alike.

CONCLUSION

The case report presented here gave the faculty a significant "teaching moment" in the dissection laboratory. It emphasized the anomaly as a valuable lifelong learning opportunity that the students could become acquainted with. The clinical cases presented an invaluable opportunity to educate aspiring physicians about the impact of malformations on affected individuals. Furthermore, studying these individuals with long life spans enables us to comprehend the crucial role of physicians in delivering effective treatment and care. This study provides an embryological elucidation of how and why the right and left vertebral arteries originate from the aortic arch and adds some historical context tracing back to the early last century with the works of Poynter (1916), Congdon (1922), Iyer (1927) and Adachi (1928). The study captures how the anomalous origin of vertebral arteries is clinically relevant in surgeries that involve the cervical region and supraaortic arch. The study also explores how the tortuosity of the proximal vertebral artery, the aortic origin of the left vertebral artery, and their impact on the hemodynamics of cerebral circulation are related. Unlike other studies that only report on this anomaly, this study delves into the clinical significance of this occurrence in other birth defects, such as Down syndrome.

ABBREVIATIONS

AA-Aortic arch; **ISA**-Intersegmental arteries; **RSCA**-Right Subclavian Carotid Artery

ACKNOWLEDGEMENTS:

The authors sincerely thank the donors and their families for their generosity, which made these studies possible and facilitated scientific and medical innovations in patient care. We are indebted to our families and the families of our donors, who inspire us to enrich and further our knowledge.

The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human donors in anatomical research.

For research using human subjects, the American Association for Anatomy endorses the protections embodied in the Basic Principles of the Declaration of Helsinki and their expansion in the regulations governing research supported by the U.S. Government (45 CFR Part 46; 56 FR 28003).

FUNDING

This study was supported by the Faculty Development for Research and Innovation grant administered by the Cooper Medical School of Rowan University.

DISCLOSURE

All authors were involved in the research and preparation process for the manuscript. CMV and Dr. Vasan performed the final part of the dissection and created the photographic images. CMV was responsible for conceiving the project idea, creating the initial draft with Dr. Vasan, and revising the manuscript with Ms. Huff until the final version was completed. Ms. Huff and Dr. Vasan conducted the literature search at the beginning, which was used to draft the manuscript and prepare the final format to be officially submitted. Ms. Huff is charged with formatting and submission of the manuscript.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Sharma, R., Sharma, R., Singla, R.K., & Kullar, J.S. (2024). A study of morphology and morphometry of scapula in North Indian population and its evolutionary significance. *Italian Journal of Anatomy and Embryology* 128(2): 31-36. https://doi.org/10.36253/ijae-15451

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

A study of morphology and morphometry of scapula in North Indian population and its evolutionary significance

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Abstract. *Introduction.* Morphometrics can quantify a trait of evolutionary significance and deduce something of their ontogeny or evolutionary relationships. The present study intends to establish the morphometric criterion of scapula in North Indians. This is of definite significance as bone morphology is known to be influenced by cultural, environmental and racial factors. *Materials and methods.* The present study was carried on 100 adult scapulae of unknown sex obtained from Department of Anatomy, Government Medical College, Amritsar. The parameters studied were length of scapula; maximum breadth of scapula; superoinferior length, transverse breadth and depth of supraspinous fossa; length of infraspinous fossa, maximum breadth of infraspinous fossa, infraspinous fossa breadth, groove for circumflex scapular vessels and its distance from lateral angle and inferior angle, ridge between origin of teres minor and teres major on lateral border of infraspinous fossa, length of attachment of teres minor on infraspinous fossa, length of attachment of teres major on infraspinous fossa; scapular index and infraspinous index. *Results.* The values of the parameters were found to be more on the right side except superoinferior length of supraspinous fossa, mean distance of the groove from the lateral angle and inferior angle. Mean scapular index and mean infraspinous index were 68.30 and 76.40 respectively. *Discussion.* The various morphologic parameters studied and morphometric values measured in our study can be used to compare the racial characteristics and study the evolutionary aspects in different populations.

Keywords: length of scapula, breadth of scapula, supraspinous fossa, infraspinous fossa, scapular index.

INTRODUCTION

The four components of the biological profile include age, sex, ancestry and stature. These four factors are important to law enforcement officials as they aid in the ability to positively identify human skeletal remains[1]. Morphometrics is a field concerned with studying variation and change in the form (size and shape) of organisms or objects. It can quantify a trait of evolutionary significance and deduce something of their ontogeny or evolutionary relationships. Also it adds a quantitative element to descriptions, allowing more rigorous comparisons[17].

Establishment of identity of an individual by studying skeletal remains has always posed a challenging problem for forensic experts[5] who are confronted with a single bone on which they are asked to give an opinion as to age, sex and stature of the individual[13].

The scapula is potentially useful in estimating stature for several reasons. First, in the absence of other intact bones, measurements taken from the scapula might be useful for estimating stature. Second, scapular measurements that can be reliably employed in estimating height are standardized and easy to locate[2]. Third, scapula might be useful in discriminant function analysis because the measurements can be taken from incomplete bones[9].

The present study intends to establish the morphometric criterion of scapula in North Indians. This is of definite significance as bone morphology is known to be influenced by cultural, environmental and racial factors.

MATERIALS AND METHODS

The material for the present study comprised of 100 adult scapulae of unknown sex, obtained from Department of Anatomy, Govt. Medical College, Amritsar. These scapulae were thoroughly cleaned and boiled and were free from any physical deformity or abrasion and were complete in all respects i.e. the upper and the lower ends were intact, so as to give the correct measurements. These were non pathological. They were labelled from 1-100 with suffix R (Right) or L (Left). The following morphological features were observed and morphometric measurements were taken.

1. **Length of Scapula:** It was measured with the help of vernier calipers from the superior angle to the inferior angle[12] (AB in Fig. 1).
2. **Maximum breadth of scapula** (Termed as **Morphological Length** by **Corruccini and Ciochon**)[3]**:** It was measured with the help of vernier calipers as the maximum distance between the middle of the dorsal border of the glenoid fossa to the end of the spinal axis at the vertebral border (AB in Fig. 2).
3. **Supraspinous fossa:**

a. *Superoinferior length* (Termed as **Supraspinous Fossa Breadth** by **Corruccini and Ciochon**)[3] : It was measured with the help of depth bar of vernier calipers as the shortest distance between superior angle and superior surface of spine of scapula (CD in Fig. 2).

b. *Transverse breadth*: It was measured with the help of vernier calipers from spinoglenoid notch to a point on the medial border where the root of spine joins it (AB in Fig. 3).

c. *Depth*: For measuring the depth of supraspinous fossa, a scale was kept touching the superior angle of scapula and upper lip of crest of spine at a point opposite to the point where lateral end of deltoid tuberosity meets lower lip of the crest of the spine. Then the maximum depth was measured with the help of depth bar of vernier calipers as shown in photograph 1 .

4. **Infraspinous fossa:**

a. *Length*: It was measured with vernier calipers from spinoglenoid notch to inferior angle (EF in Fig.2).

b. *Maximum breadth:* It was measured with the help of vernier calipers from infraglenoid tubercle to root of spine (AG in Fig.2).

c. *Infraspinous fossa breadth*: It was measured as distance between inferior angle and midpoint of attachment of spine on medial border of scapula [16].

d. *Groove for circumflex scapular vessels*: It was observed on lateral border of infraspinous fossa. If demarcated, its distance from lateral and inferior angles were measured with the vernier calipers (HI & JK in Fig.2).

e. *Ridge between origin of teres minor and teres major on lateral border of infraspinous fossa*: It was observed for its presence or absence an if present whether it was inclined obliquely or transversely.

f. *Length of attachment of teres minor on infraspinous fossa*: It was measured with the help of vernier calipers (LM in Fig.2).

g. *Length of attachment of teres major on infraspinous fossa:* It was measured with the help of vernier calipers (NO in Fig.2).

5. **Scapular index : breadth/length *100**
6. **Infraspinous index : Breadth/infraspinous length*100**

RESULTS

1. Length/Height of bone

The mean length of scapula was found to be 140.55 mm (Range = 111.14 -162.58 mm) with 143.02 mm (Range = 112.97 – 162.58 mm) on the right side and 138.08 mm (Range = 111.14 – 158.51 mm) on the left side. When compared on both the sides, the length was larger on the right side as compared to the left side (Table 1).

Table 1.

Parameter	Mean (mm)			Range(mm)		
	R	L	Total	R	L	Total
Length of scapula	143.02	138.08	140.55	112.97-162.58	111.14-158.51	111.14-162.58
Breadth of scapula	96.79	95.22	96.00	84.19-114.66	82.69-110.54	82.69-114.66
Length of medial border	162.36	157.20	159.78	129-193	131-186	129-193
Supraspinous fossa						
a)Length	31.75	32.76	32.25	18.15-42.72	11.79-45.44	11.79-45.44
b)Transverse breadth	81.39	81.60	81.50	64.72-96.53	67.85-96.11	64.72-96.53
c)Depth	18.83	16.51	17.67	12.69-26.42	9.82-25.42	9.89-26.42
Infraspinous fossa						
a) Length	130.29	126.97	128.63	111.10-147.57	100.18-142.90	100.18-147.57
b) Maximum breadth	97.99	98.55	98.27	77.98-111.31	82.14-138.25	77.98-138.25
c) Breadth	106.38	103.12	104.75	80.59-119.32	85.40-126.64	80.59-126.64
d) Distance of groove from lateral angle	22.79	24.98	23.88	19.90-54.03	14.16-45.94	14.16-54.03
e) Distance of groove from inferior angle	61.85	62.57	62.21	74.70-110.14	69.57-113.96	69.57-113.96
f) length of attachment of teres minor	50.51	40.77	45.64	25.97-92.75	46.87-77.72	25.97-92.75
f) length of attachment of teres major	33.76	24.35	29.06	19.24-82.36	23.02-58.78	19.24-82.36
Scapular index	67.78	68.96	68.30			
Infraspinous index	75.21	77.62	76.40			

2. Maximum breadth

The mean breadth was found to be 96.00 mm (Range = 82.69 – 114.66 mm). On the right side it was found to be 96.79 mm (Range = 84.19 – 114.66 mm) and on the left side, it was found to be 95.22 mm (Range = 82.69 – 110.54 mm). On comparing both the sides, the mean breadth was more on the right side as compared to the left side (Table 1).

3. Supraspinous fossa

(a) Superoinferior length (supraspinous fossa breadth)

The mean for superoinferior length of supraspinous fossa (supraspinous fossa breadth) was found to be 32.25 mm (Range = 11.79 – 45.44 mm). On the right side, it was observed to be 31.75 mm (Range = 18.15 – 42.72 mm) and on the left side, it was found to be 32.76 mm (Range = 11.79 – 45.44 mm). Thus it was slightly greater on the left side than on the right side (Table 1).

(b) Transverse breadth (supraspinous fossa)

The mean value found for the transverse breadth of supraspinous fossa was 81.50 mm (Range = 64.72 – 96.53 mm). It was 81.39 mm (Range = 64.72 – 96.53 mm) on the right side and 81.60 mm (Range = 67.85 – 96.11 mm) on the left side (Table 1 and 4).

(c) Depth of supraspinous fossa

The mean depth of supraspinous fossa was found to be 17.67 mm (Range = 9.89 – 26.42 mm). On the right side it was found to be 18.83 mm (Range = 12.69 – 26.42 mm) and on the left side it was 16.51 mm (Range = 9.82 – 25.42 mm). Thus it was found to be more on the right side than the left side (Table 1).

4. Infraspinous fossa

(a) Length of infraspinous fossa from spinoglenoid notch to inferior angle

Its mean value was found to be 128.63 mm (Range= 100.18 – 147.57 mm). On the right side it was seen to be 130.29 mm (Range=111.10 – 147.57 mm) and on the left side it was 126.97 mm (Range = 100.18 – 142.90 mm). Thus it was seen that the values for the right side were higher than those for the left (Table 1).

(b) Maximum breadth of infraspinous fossa

The mean value for maximum breadth of infraspinous fossa was found to be 98.27 mm (Range = 77.98 –

138.25 mm). It was found to be 97.99 mm (Range = 77.98 – 111.31 mm) on the right side and 98.55 mm (Range= 82.14 – 138.25 mm) on the left side. So it was seen that the value for the left side were more than the right side (Table 1).

(c) Infraspinous fossa breadth

The mean value for infraspinous fossa breadth was observed to be 104.75 mm (Range = 80.59 – 126.64 mm). The mean observed on the right side was 106.38 mm (Range = 80.59 – 119.32 mm) and on the left side was 103.12 mm (Range = 85.40 – 126.64 mm). Thus the values on right side were more than the left side (Table 1).

(d) Groove for circumflex scapular vessels

The groove for circumflex scapular vessels was distinct in 33 (66%) bones and indistinct in 17 (34%) bones on the right side. It was distinct in 35 (70%) bones and indistinct in 15 (30%) bones on the left side.

The mean distance of the groove from the lateral angle was observed to be 23.88 mm (Range = 14.16 – 54.03 mm). On the right side, it was 22.79 mm (Range = 19.90 -54.03 mm) and on the left side, it was 24.98 (Range = 14.16 – 45.94 mm). Thus the value for left side was more than the right side.

The mean distance of the groove from the inferior angle was observed to be 62.21 mm (Range = 69.57 – 113.96 mm). It was observed to be 61.85 mm (Range = 74.70 – 110.14 mm) on the right side and 62.57 mm (Range = 69.57 – 113.96 mm) on the left side. Thus the value for this parameter was also more for the left side.

Thus it was seen that the distance of the groove was more from the inferior angle than the lateral angle.

(e) Ridge between origin of teres minor and major

On the right side, it was observed to be present in 38 (76%) bones and absent in 12 (24%) bones while on the left side, it was present in 32 (64%) bones and absent in 18 (36%) bones. Out of these, in majority, it was obliquely inclined (35 on the right side and 27 on the left side) while in the rest (3 on right side and 5 on left side), it was transversely inclined. Standring et al[15] has also mentioned this ridge to be obliquely inclined.

(f) Length of attachment of teres minor

The mean length of attachment of teres minor was found to be 45.64 mm (Range = 25.97 – 92.75 mm). On the right side it was 50.51 mm (Range = 25.97 – 92.75 mm) while on the left side it was 40.77 mm (Range = 46.87 – 77.72 mm). Thus the length occupied by teres minor was much more on the right side as compared to the left side (Table 1).

(g) Length of attachment of teres major

The mean length of attachment of teres major was observed to be 29.06 mm (Range = 19.24 – 82.36 mm). On the right side it was 33.76 mm (Range = 19.24 – 82.36 mm) while on the left side it was 24.35 mm (Range = 23.02 – 58.78 mm). Thus the length occupied by teres major was much more on the right side as compared to the left side (Table 1).

From the above two parameters it can be inferred that the length of attachment of teres minor on scapula is much more than that of teres major.

5. Scapular index

Mean scapular index was seen to be 68.30. It was observed as 67.68 on the right side and 68.96 on the left side (Table 1).

6. Infraspinous index

Mean infraspinous index was observed to be 76.40. It was observed as 75.21 on the right side and 77.62 on the left side (Table 1) .

DISCUSSION

Table 2 compares length of scapula in different races as mentioned in the literature. Von Schroeder et al[16], Piyawinijwong et al[12], Coskun et al[4], Ozer et al[10] (maximum scapular height), Paraskevas et al[11], Singhal et al[14], Krishnaiah et al[8], and Jacinth and Vikraman[7] studied the parameter before and there were some variations in their results. These variations could be attributed to racial factors. Our results are in consonance with those of Singhal et al[14].

Earlier Von Schroeder et al[16], Piyawinijwong et al[12], Singhal et al[14], Krishnaiah et al[8], and Jacinth and Vikraman[7] had measured the maximum breadth of scapula (Table 3). Our results were in consonance with those of Piyawinijwong et al[12], Singhal et al[14] and Jacinth and Vikraman[7].

Coskun et al[4] also worked upon the superoinferior length (supraspinous fossa breadth) and reported that mean length was 43 mm (Range = 24.6-58.4). Our values were less than those found by them. This could be attributed to racial factors.

Von Schroeder et al[16] and Piyawinijwong et al[12] measured transverse breadth of supraspinous fossa. The results observed in our study were more than Piyawinijwong et al[12] but less than Von Schroeder et al[16].

Table 2. Showing comparison of maximum length of human scapula.

Authors	Race	Mean (n) (mm)		Range(mm)	
		Right	Left	Right	Left
Von Schroeder et al[8]	Canadian	155 (30)		127-179	
Piyawinijwong et al[12]	Thais	131.1 (97)		115.7-159.6	
Coskun et al[4]	Turkish	98.8(90)		76-115	
Ozer et al[10]	Anatolian	144.41(186)			
Paraskevas et al[11]	Greek	147.6 (88)		129-168	
Singhal et al[14]	Gujarati	141.7			
Krishnaiah et al[8]	Nalgonda	143.27			
Jacinth and Vikraman[7]	Tamil	133			
Present study	North Indian	140.55 (100)		111.14 – 162.58	
		143.02(50)	138.08(50)	112.97-162.58	111.14-158.51

Table 3. Showing comparison of maximum breadth of human scapula.

Authors	Race	Mean (n) (mm)		Range (mm)	
		Right	Left	Right	Left
Von Schroeder et al[16]	Canadian	106.0 (30)		92-122	
Piyawinijwong et al[12]	Thais	95.7 (97)		86.6-114.7	
Singhal et al[14]	Gujarati	96.4			
Krishnaiah et al[8]	Nalgonda	105.6			
Jacinth and Vikraman[7]	Tamil	96.25			
Present study	North Indian	96.00		82.69-114.66	
		96.79(50)	95.22(50)	84.19-114.66	82.69-110.54

Table 4. Showing comparison of transverse breadth of supraspinous fossa.

Authors	Race	Mean (n) (mm)		Range (mm)	
		Right	Left	Right	Left
Von Schroeder et al[16]	Canadian	85.5 (30)		71-101	
Piyawinijwong et al[12]	Thais	76.2 (97)		70-90	
Present study	North Indian	81.50		64.72-96.53	
		81.39(50)	81.60(50)	64.72-96.53	67.85-96.11

Earlier Krishnaiah et al[8], and Jacinth and Vikraman[7] found the length of infraspinous fossa from spinoglenoid notch to inferior angle to be 107.71mm and 105.3 mm respectively. Our results observed larger length of infraspinous fossa than theirs.

Previously Coskun et al[4] have measured infraspinous fossa breadth and found it to be 113.5 mm (Range = 79.8-135.4 mm). The results of present study were less than theirs. This could be attributed to racial factors.

Singhal et al[14], Krishnaiah et al[8], and Jacinth and Vikraman[7] have calculated scapular index to be 68.5, 73.99 and 72.6 respectively. Our result was in consonance with Singhal et al[14]**.** They calculated infraspinous index as 94.6, 98.33 and 91.72 respectively. Our result was not in consonance with any of them.

The erect posture of humans has initiated many morphologic changes in the evolution of upper limb especially scapula, due to increase in functional demands of a prehensile limb. From massive scapula with laterally pointing glenoid, screw-shaped articular surface in Rachimatous amphibians to its relocation to a lower place in Reptilia, scapula underwent many changes. Slowly, it was noticed that glenoid shifted from lateral to posterior and inferior position; scapular spine appeared and coracoid was reduced to coracoid process. The scapular shape depends on posture and functional requirements of muscles which are attached to it. It is similar in humans and those mammals which have partially or completely freed the pectoral limbs. These changes were brought as a result of change in posture from pronograde to orthograde and specialization as a prehensile limb. In pronogrades, the scapula is long and narrow whereas it becomes broader in man (especially seen in primates). The part of scapula below the spine shows the most conspicuous changes. The scapular index is seen to be higher in pronogrades than orthogrades, because of gradual increase in scapular breadth and increase in length of bone below the level of spine and hence, 'infraspinous index' significantly[7]. The change in length of scapula below the spine has also been reported by Inman, Saunders and Abbott, which is significant in mechanism of shoulder[6]. The various morphologic parameters studied and morphometric values measured in our study can be used to compare the racial characteristics and study the evolutionary aspects in different populations.

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IJAE
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Firenze University Press
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OPEN ACCESS

Citation: Galassi, F.M., Varotto, E., Carotenuto, G., & Sineo, L. (2024). Anatomical and radiological notes on the sternum of Sicily's earliest known woman (San Teodoro Cave, Messina, Palaeolithic Period, 14,500 BP). *Italian Journal of Anatomy and Embryology* 128(2): 37-40. https://doi.org/10.36253/ijae-15467

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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Anatomical and radiological notes on the sternum of Sicily's earliest known woman (San Teodoro Cave, Messina, Palaeolithic Period, 14,500 BP)

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Abstract. In this brief anatomical and palaeopathological communication the authors detail the characteristics and alterations noted on the sternal body and xiphoid process of ST1, Sicily's earliest known female skeleton, dated to the Palaeolithic Period (14,500 years BP). A morphological and radiological analysis is offered both in terms of the relation between age and manifestation of the xiphisternal fusion, with a focus on ancient traumatology involving the soft tissues near the sternum to explain calcification of xiphoid and, more likely, the local presence of an exostosis.

Keywords: anatomy, anthropology, heterotopic calcification, radiology, sternum, xiphoid process.

INTRODUCTION

The San Teodoro Cave (*Grotta di San Teodoro*, Acquedolci, Messina, North-Eastern Sicily) has been the subject of considerable anthropological, zooarchaeological and ecological interest ever since its first exploration by Baron Francesco Anca di Mangalaviti (1803-1887) in 1860 since it represents the earliest known evidence of the peopling of the Mediterranean island of Sicily. This site (Fig. 1a,b) became even more interesting when, from 1937 on, human skeletons started to be excavated leading to the discovery of remains of seven individuals (ST1 to ST7). Individuals ST1-5 were described by Graziosi in 1947 (Graziosi 1947), while the fragmentary remains of the skull of ST6 were later described by Pardini in 1975 (Sineo et al. 2002; D'Amore et al. 2009). In 1989, Aimar and Giacobini published their skeletal assessment of ST7 (Sineo et al. 2002; D'Amore et al. 2009), whereas, in 1993, Fabbri proposed a morphometric revision of sex and stature of ST1 and ST4 (Fabbri 1993). In this research we focused on ST1, a young adult individual (originally considered to be male based on the cranial morphology, later proposed to be a female based on pelvic morphology), radiocarbon-dated to 14,500 BP, whose osteological remains (Fig. 1c) are currently exhibited in Palermo's G.G. Gemmellaro Museum of Palaeontology. At present, a novel thorough multidisciplinary reassessment of the skeletal remains of this individual – popularly nicknamed "Thea" – is taking place under the scientific leadership of the University of Palermo (Prof. L. Sineo). In the context of this global analysis, special attention has been paid to ST1's thorax and especially the morphology of her sternum by offering an anatomical and radiological perspective.

MATERIALS AND METHODS

The sternum, which could be assessed through direct visual inspection in the said museum, only consisted of the body of the sternum and the xiphoid process, while the manubrium was not available for this study since it has not yet been "repatriated" to Sicily to this date and is still currently housed in the Florentine Museum and Institute of Anthropology. Beside the gross morphological examination, the study was complemented by Computerised Tomography (CT) scan imaging analysis and a 3D digital reconstruction. CT scanning of the sternum was performed using a General Electric LightSpeed VCT 64 Slice CT multidetector scanner with a gantry rotation time of 0.6 s, a slice thickness of 0.625 mm, and maximum intensity projection for integration. Images were saved (as bitmap files) in Digital Imaging and Commutations in Medicine format and processed, visualised, and segmented using the open-source software 3D Slicer (Fedorov et al. 2012). Finally, a differential diagnosis has been proposed and an anatomical contextualization presented as is customary in palaeopathological studies (Buikstra 2017; Galassi et al. 2020; Varotto et al. 2021).

RESULTS

ST1's sternum, in both the anterior and posterior views (Fig. 1d,e), shows a complete synostosis of the xiphi-

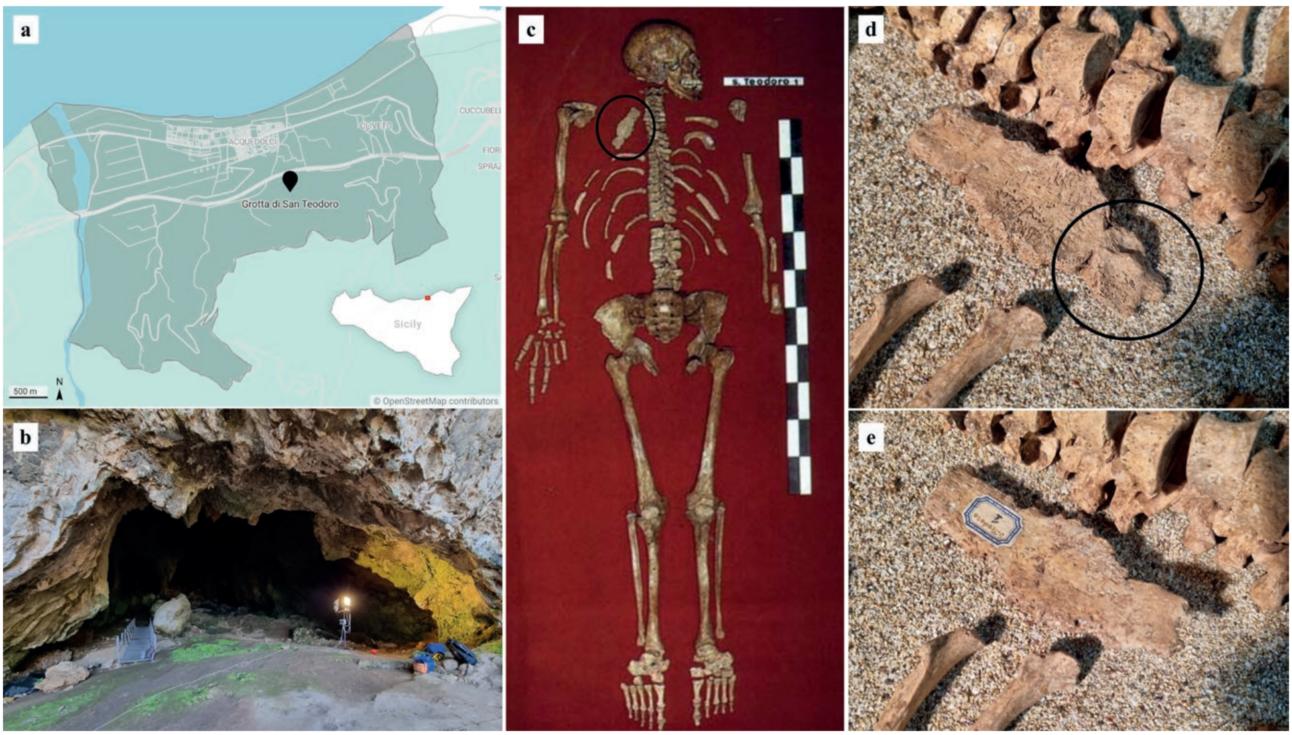

Figure 1. a. The geographical position of the San Teodoro Cave in the territory of the municipality of Acquedolci (province of Messina, Sicily) – map made by coauthor E.V. with Datawrapper (www.datawrapper.de); **b**. The entrance of the cave during scientific explorations (image by co-author and project leader L.S.); **c**. ST1's skeleton displayed in the G.G. Gemmellaro Museum of Palaeontology (Palermo, Italy), with the sternum highlighted by a black circle – image by the Museum in the public domain; **d**. anterior view of ST1's body and xiphoid process. The investigated structure lies within a black circle; **e**. posterior view of the sternum.

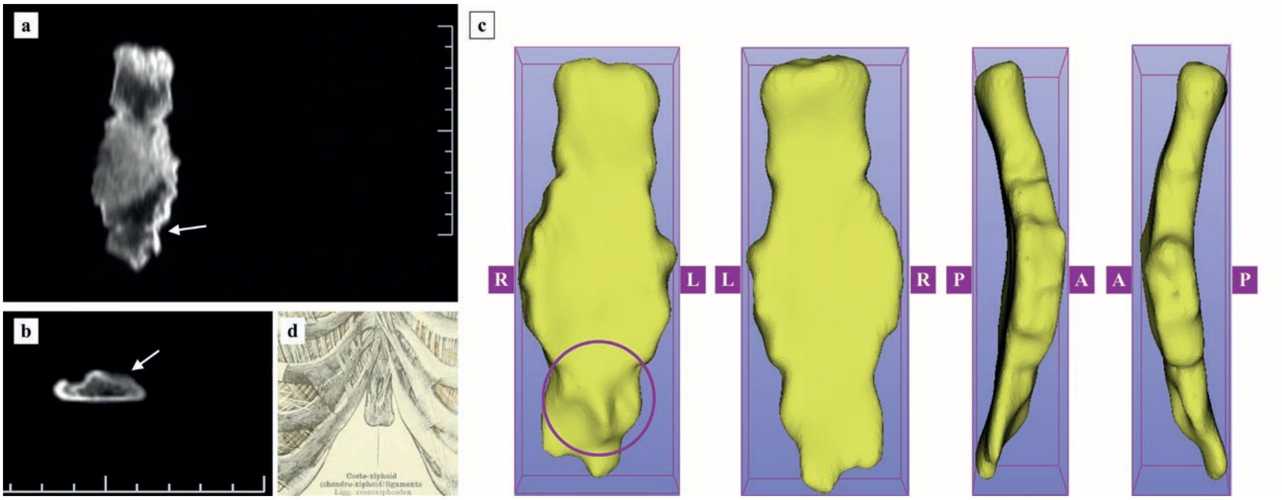

Figure 2. a. Coronal and **b**. transverse sections of ST1's sternum (ST1's sternum) – white arrows indicate the investigated structure; **c**. the four views (left to right of the image: anterior, posterior, right lateral and left lateral) of the sternum in a 3D digital reconstruction. The purple circle in the first image highlights the heterotopic calcification and exostosis; **d**. schematic anatomy of the ligaments of the human sternum from C. Told's *An Atlas of Human Anatomy for Students and Physicians* (1919), copyright expired.

sternal joint and, even more patently in the anterior view (Fig. 1d) a heterotopic calcification of the entire xiphoid process. Additionally, the anterior surface of the sternum (Fig. 1d) shows an uncommon outgrowth of bone that can be appreciated perpendicularly to the coronal plane. This evidence is corroborated by the computed tomographic examination of the internal bone structure (Fig. 2a,b). A 3D digital reconstruction of the fused sternal body and xiphoid process was provided in the four views (Fig. 2c).

DISCUSSION

With reference to the xiphisternal fusion, it has been observed that it starts between 30 and 39 years of age and can be confidently used to predict age at death in forensic anthropological studies (Partido Navadijo & Alemán Aguilera 2022). This could partly be compatible with the ST1's estimated age category as the young adult one (20-35 yrs) but it should be underlined that this phenotype can be either the result of a congenital anomaly or merely a normal manifestation during an individual's growth.

Moreover, with reference to the described exostosis emerging from the heterotopic calcification of the xiphoid process, through the CT scan analysis, it was possible to exclude that it was the result of the healing process following a bone fracture because of the absence of fracture lines or of a congenital anomaly, since no cases with a comparable morphology and topography are known to have been reported so far.

For this reason, a soft tissue trauma cannot be excluded *a priori* from the potential aetiologies in question. This scenario could indeed be compatible with a subsequent heterotopic calcification of the chondroxiphoid ligaments (Fig. 2d), which is nonetheless more rarely observed in individuals under 45 years of age (Duraikannu et al. 2016), it being very rare in 20-year-olds – and in the present-day clinical setting occasionally seen only after locoregional surgery (Hong et al. 2016).

CONCLUSIONS

The simultaneous presence of synostosis of the xiphisternal joint with evidence of a heterotopic calcification of the whole xiphoid process and the contextual heterotopic calcification of the chondroxiphoid ligaments in a young adult such as ST1 represents an element to be considered in the general state of health of Sicily's earliest known woman and a valuable insight into the evolution and adaptation of sternal anatomy to palaeotraumatological events in past human individuals, well before the dawn of civilisation and the birth of thoracic surgery.

While future research could be directed at the assessment of sternal morphologies, both normal and pathological, in a larger Sicilian and Italian skeletal sample, this study's immediate goal is also to assist researchers in distinguishing age-related calcification of skeletal tissue from the secondary effects of soft tissue trauma.

ACKNOWLEDGMENTS

A preliminary version of this research was presented as a poster at the 25th National Congress of the Italian Anthropological Association (AAI), Turin, 6th-8th September 2023. Due to the archaeological nature of the examined skeletal remains, no contemporary patient consent form could be applied to this case; nonetheless, all Italian national laws have been followed during this research as well as the relevant authorisations have been obtained. The authors thank Curator Carolina Di Patti and Director Alessandro Incarbona, from the G.G. Gemmellaro Museum of Palaeontology, for their permission to study the remains in question as well as numerous colleagues who have offered their technical assistance with this investigation.

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Citation: Ciccarelli, A., Forte, F., Pindinello, I., Cofone, L., Galassi, F.M., Circosta, F., Papa, V., & Taurone, S. (2024). Human coronary vessels: Distribution of cholinergic nerve fibres and age-related changes. *Italian Journal of Anatomy and Embryology* 128(2): 41-48. https://doi.org/10.36253/ijae-15490

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Human coronary vessels: Distribution of cholinergic nerve fibres and age-related changes

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Abstract. *Background*. Cholinergic nerve fibres were studied in the human coronary vascular tree by means of acetylcholinesterase staining and choline acetyltransferase activities on autopsy samples of coronary vessels (arteries, veins, and micro-vessels). *Methods*. Samples of human coronary vessels were harvested in younger and older subjects. Samples were exposed to the enzymatic and or immune staining for acetylcholinesterase and choline acetyltransferase, two enzymes linked to the metabolism of acetylcholine. The morphological data were subjected to the quantitative analysis of images and to the statistical analysis of data. *Results*. Both acetylcholinesterase and choline acetyltransferase activities are localised in the human coronary vessels. Structures resembling cholinergic nerve fibres are located in the extra-parenchymal and intra-parenchymal branches of these vessels. *Discussion and Conclusions*. The quantitative analysis of images and statistical analysis of data demonstrate that the cholinergic innervation of coronary vessels (especially the extra-parenchymal branches) is well represented. Moreover, in older subjects both the enzymes are strongly decreased. The extra- and intraparenchymal branches of the human coronary arteries and veins are provided with cholinergic nerve fibres, which could control the efferent sensitive pathways and the autonomic nerve fibres of the coronary vascular tree.

Keywords: coronary vessels, Cholinergic Nerve Fibres (CNF), acetylcholinesterase (AChE), choline acetyltransferase (ChAT), histochemistry, age-related changes.

INTRODUCTION

Heart failure (HF) is one of the most common chronic pathologies in the elderly population and is characterised by a decrease in left ventricular function and biohumoural changes such as hyperactivation of the sympathetic system, the renin-angiotensin-aldosterone system and the inflammatory system (Hunt et al., 2009). The prevalence of HF in the general population has been estimated to be between 0.4 and 2%. In Italy about 2 million patients are affected by HF and more than a third of these do require an average annual hospitalisation involving a significant cost for the national health system (Rego et al., 2004).

The hyperactivity of the Sympathetic Nervous System (SNS) observed in heart failure initially represents an adaptation process aimed at compensating for the reduction in cardiac performance; however, the chronic increase in plasma CA is associated with a marked dysregulation of cardiac β-adrenergic receptors both at the receptor and post-receptor levels, responsible for a structural damage characterised by: ventricular hypertrophy, focal necrosis, inflammation and increased collagen deposition resulting in myocardial interstitial fibrosis. Furthermore, chronic HF is associated with a progressive loss of both adrenergic and cholinergic cardiac nerve fibres.

Cardiac tissue is extensively innervated by the autonomic nervous system, which is characterised by the presence of sympathetic and parasympathetic fibres. Sympathetic neurons release norepinephrine, which acts post-synaptically on the β1-AR and β2-AR receptors, whereas parasympathetic fibres release acetylcholine which acts on muscarinic receptors. The integration of sympathetic and parasympathetic fibres and their respective neurotransmitters is capable of modulating heart rate (chronotropic function), electrical impulse conduction speed (dromotropic function), myocardial relaxation (lusitropic function) and myocardial contraction (inotropic function) (Kapa et al., 2016).

Studies in the literature report that the density of cardiac nerve fibres decreases by up to 50% with age and is found to be altered in numerous pathological conditions (e.g. myocardial infarction) (Hopkins et al., 2000), in which cell proliferation occurs following Wallerian degeneration and disorganised axonal regeneration with consequent repercussions on cardiac function.

Despite numerous studies, cholinergic innervation in the human coronary vascular district has not been completely clarified (Hunt et al., 2009). Different animal species, in fact, have been showing different answers to acetylcholine (AChE). In particular, this enzyme is able to induce vasodilatation and vasoconstriction (Wang et al., 2006) in isolate preparations of coronary arteries, with the key role of endothelial cells (Rengo et al., 2004), through stimulation of muscarinic receptors (Kapa et al., 2016) and smooth muscle cells fibres. Moreover, an intra-coronary injection of acetylcholine in living humans produces an increase of the coronary circulation rate, hence a strong vasodilatation.

To explain these apparent contradictions, acetylcholine was supposed to induce vasodilatation in small coronary branches, while vasoconstriction of the large calibre extra-parenchymal branches (Bakovic et al., 2013; Hirsch & Kaiser, 1971, Staoyanou et al., 2021).

Subsequent studies on the nervous regulation of coronary circulation and on the cholinergic neuromodulation of the coronary vessels have failed to agree with one another (Ludmer et al., 1986; Armour, 2011).

Therefore, it was opted to carry out the present study to assess the presence in the human coronary vessels of acetylcholinesterase (AChE) and/or choline acetyltransferase (ChAT) positive nervous fibres, and their distribution in younger and older individuals.

MATERIALS AND METHODS

All procedures were in accordance with the ethical standards of the Declaration of Helsinki (1964) of the World Medical Association (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). All the photographs were made before 1979, when written informed consent was not available. Samples of coronary arteries, veins, and heart parenchyma (for the studies of the coronary microcirculation) were harvested, during autopsies performed 24-36 hours after death in 24 individuals of both sexes, whose ages had an average of 26 years in n= 8 subjects and of 76 in n= 16 subjects.

It has been previously demonstrated by More and Fatty (Moore & Fatty, 1958) that cholinesterase activity remains stable in tissues for as long as 24-48 hours after death, so that an autopsy series can be used also for immunochemical staining. The whole diameter of each vessel was measured with a calibrated gauge and all vessels were collected with the following calibre class (vd. Rhodin, 1967): 1) extra-parenchymal branches of large capacitance with diameter > 500 µm; 2) intra-parenchymal arteries of large calibre with diameter >150 µm; 3) arteries of middle calibre with diameter between 50-150 µm; 4) arterioles of small calibre with diameter between 35-50 µm; 5) micro-vessels with a diameter between 10-35 µm 6) capillaries with a diameter < 10 µm; 7,8 and 9) veins of small, middle and large calibre (with the same diameter as the related class of arteries).

Capillaries are made by only the endothelial layer. The vessels of middle calibre possess endothelial and adventitial layer, while the larger vessels possess three layers (endothelial, middle smooth muscle and adventitial). After harvesting, the samples were washed for 30-60 minutes at 4°C in an isotonic Na Sulphate solution and subsequently submitted to the following procedures : 1) either frozen and serially cut under a refrigerated microtome in order to obtain 8-10mm thin slices; 2) or paraffin embedded and serially cut in slices of 4-5μm, in transversal thin sections for the entire length of the vessel. These samples are considered as whole-mount and include all the thickness of the wall of the vessel; 3) other samples were dissociated under an operative microscope with the aid of a micro-scissor, into thin sheets, corresponding with the three normal components of the vascular wall (intima, media and/or adventitia). The samples 2 and/or 3 were made transparent by adding a drop of glycerol and mounted on glass slides for light microscopy. The sample 1 was vacuum-dried and dehydrated for 60 minutes in a P_2O_5 gaseous atmosphere. Then all the samples were submitted to: 1) Bodian's morphological method for the staining of all the types of nerve fibres; 2) enzymatic method for the staining of acetyl-cholinesterase (AChE) (this method stains AChE-positive nerve fibres); 3) immuno-histochemical method for choline acetyltransferase (ChAT) (this method stains specifically cholinergic nerve fibres); 4) quantitative analysis of images; 5) statistical analysis of the data.

1) Staining of all nerve fibres. All the nerve fibres were stained using the Bodian's method (Bodian, 1936). This method can be used to verify that a stained structure is nervous in nature. Indeed, it stains all nerve fibres and neuro-fibrils. After fixation, the sections were treated with: i. 1% Protargol solution (colloidal silver), ii. reducing solution (Hydroquinone + sodium sulphite), iii. 1% Gold chloride solution, iv. 2% oxalic acid solution and counterstained with 0.03% aniline blue. The nerve fibres and neuro-fibrils are stained in black.

2) AChE staining: The slices were incubated in a buffer phosphate solution containing 0.5 mM acetylcholine iodide (Koelle, 1963) in the presence of cholinesterase specific inhibitor iso-OMPA 10-6 mole/litre (Du Bois et al., 1950). The reaction lasted 30-60 min (short time). The controls were performed either avoiding the substrate or adding the specific inhibitor (Karnowsky & Root, 1964)

The addiction of eserine (specific inhibitor) to incubation medium or the lack of iodide acetylcholine substrate caused the negativity of the reaction expressed by not coloured CNF. The sections were washed in a cold phosphate buffer, stained for haematoxylin-eosin as contrast, mounted in Entellanâ (Merck) and observed under light microscopy.

3) ChAT staining: Transverse cryostat sections of our samples (20-40 m) were mounted on glass slides and allowed to dry at room temperature. The sections were then incubated at room temperature with anti-ChAT monoclonal antibodies Lyophilised Monoclonal (NCL-ChAT) Clone 38B12 (Novocastra) was used diluted 1:50 as indicated by the producer. Choline acetyltransferase (ChAT) is a 68kD enzyme which catalyses the synthesis of acetycholine (ACh) from choline and acetyl coenzyme A. The human ChAT gene encodes two proteins, the 68kD ChAT enzyme and a 27kD protein immunologically related and co-expressed with ChAT in cholinergic neurons of the central and peripheral nervous system. The smaller proteins may play a role in the regulation of ACh synthesis. NCL-ChAT specifically labels central and peripheral cholinergic neurons. NCL-ChAT does not label some central axons in the insular cortex or in the internal capsule, non-cholinergic structures, endothelial cells and microglia (Novocastra Catalog 2003). ChAT is the key enzyme for the turnover of acetylcholine (Perse, 1972). Slides were then rinsed and incubated each with 1 ml, diluted 1:200 in phosphate buffer, of a biotinylated goat anti-rabbit secondary antibodies. Sections were then incubated with an avidin-biotin-peroxidase complex, diluted 1:200 in phosphate buffer, (K3468, Dako Corporation, Carpentera, CA, USA) for 20 min, developed in acetate-imidazole buffer (containing 0.25 % nickel sulphate, 0.04 % diamino-benzidine, and 0.005 % hydrogen peroxide) counterstained with haematoxylin – eosin, dehydrated and mounted. Negative controls were performed with iso-type matched irrelevant antibodies, while positive controls were made with specific antisera raised against peripheral ChAT. All samples, without any selection, were stained, observed, photographed, counted by quantitative analysis of images, and submitted to statistical analysis. On the contrary, the images were selected in a limited number and are representative of the tissue sample in general. Only these images have been described in our morphological results, while the general results are comprehensive also of the findings emerging from all the observed images. Observations and photographs were performed with a photo-microscope Carl Zeiss (Jena Germany) PMQ II.

4) Quantitative analysis of images: A quantitative analysis of the intensity of the staining was performed by means of a Quantimet Analyser (Leicaä), provided with specific software including internal controls. The values coming from samples incubated without substrate were considered as 'zero'. The values reported in our experiments represented the intensity of staining for each type of vessel and are expressed as Conventional Units (C.U.) ± standard error of the mean; further detailson QAI and on definition of CU are reported in the Manual of the Quantimet Leica 2000 image analyser (Manual of methods: Quantimet Leica 2000).

5) Statistical analysis of data: To ascertain the significance of QAI, it is mandatory to perform a statistical analysis including basic statistical methods such as: mean values, maximum and minimum limits, variations, Standard Deviation (SD), Standard Error of Mean (SEM), probability index (p) and Student's t-test. The majority of these data were calculated, but not tabled (only SEM and p are tabled). All these statistical results demonstrate a high significance of our morphometrical data (Castino & Roletto, 1992).

RESULTS

In stretched flat on slides or in the serial sections of the left and right coronary arteries as well as of anterior and posterior interventricular branches we can observe AChE positive structures that had been identified as cholinergic nervous fibres (CNF). CNF have been observed in all three layers of the coronary arteries (adventitia, media, and intima) and in both times of incubation used (short and long times). The addiction of eserine (specific inhibitor) to incubation medium or the lack of iodide acetylcholine substrate caused the negativity of the reaction expressed by not coloured CNF. Comparing the figures 1 and 2 stained for AChE with short and long times of incubation we can see that CNF were organized in a plexus localized in outer adventitia zone. We can observe a strong increase of colouring with long times of incubation. Figures 3 and 4 are stained for ChAT with short and long times of incubation.

Table 1. Variations of the cholinergic nerve fibres in the human coronary vessels.

Coronary vessels	Subjects (n=12)
Large calibre coronary extraparenchymal branches*	19.3±1.6
Small calibre coronary extraparenchymal branches*	4.2±1.4**
Coronary veins*	1.5±0.16**

* All results are expressed in conventional unit (=CU) ± SEM.

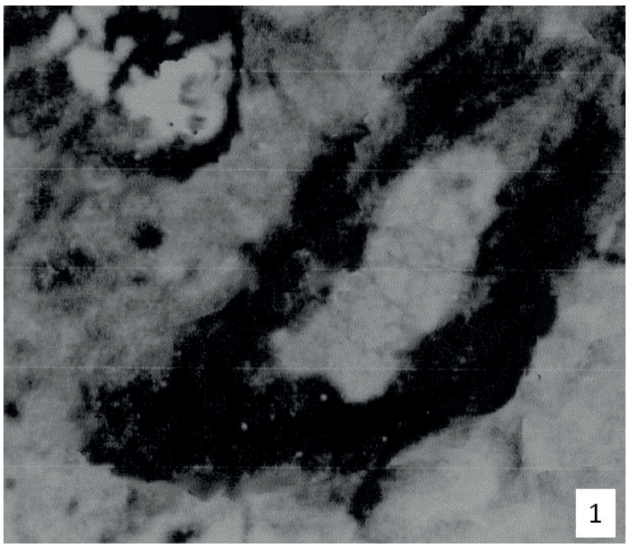

Figure 1. Full-thickness preparation of adventitia of a large intraparenchymal branch of the right coronary artery of a 57-year-old male individual. AChE activity after a short incubation time. Note the presence of staining in the adventitia of the vessel. (Original magnification: 450 x; calibration bar 10 mm). Image made in 1978.

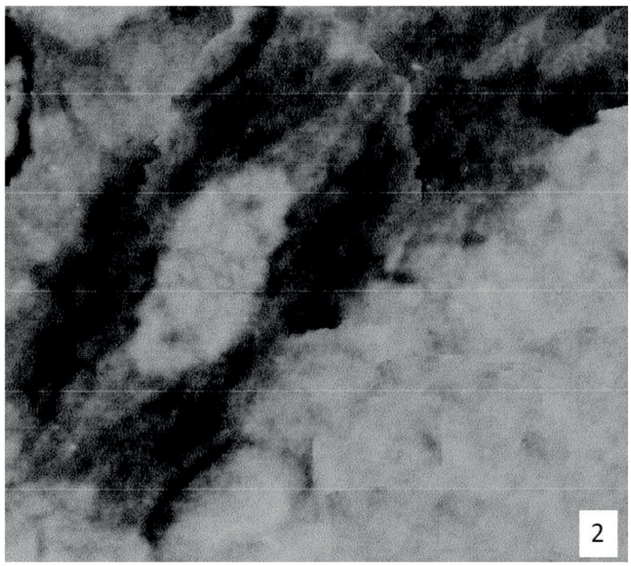

Figure 2. Full-thickness preparation of adventitia of a large intraparenchymal branch of the right coronary artery of the same subject as in Fig. 1 (serial section). AChE activity after a long time of incubation. We can observe a strong increase of the staining in the adventitia of the vessel. (Original magnification: 450 x; calibration bar 10 mm). Image made in 1978.

Table 2. Regional variations of the AChE-positive nerve fibres in the human coronary vessels after 1h or 6h of incubation.

Human coronary vessels	Incubation 1h	Incubation 6h
Left coronary artery (adventitia)*	29.3 ± 1.6	70.1 ± 0.9**
Posterior intra-ventricular branch*	25.2 ± 1.4	67.3 ± 0.6**
Microcirculation (arterioles and capillaries)	0	0
Small intraparenchymal branch (whole thickness transversal section)*	31.5 ± 1.6	82.4 ± 0.6**

* All results are expressed in conventional unit (=CU) ± SEM** P = 0.001 incubation 6h vs 1h.

Table 3. Age-related changes of the AChE-positive nerve fibres in the human coronary vessels.

Human coronary vessels	Young (n=8)	Old (n=16)
Large extra-parenchymal branches*	19.3 ± 1.6	10.1 ± 0.9**
Small intra-parenchymal branches*	14.2 ± 1.4	7.3 ± 0.6**
Microcirculation (arterioles and capillaries)	0	0
Large and Small veins*	11.5 ± 1.6	5.4 ± 0.6**

* All results are expressed in conventional unit (=CU) ± SEM.
** P = 0.001 older vs younger.

The results confirm the same observation as in figures 1 and 2. Great arterial branches appeared more diffusely innervated than smaller branches. In the larger arteries the periadventitial plexus is formed by thick CNF (from 20 to 30 μm in diameter) and by a few thin CNF (Fig. 1,2). In the smaller arteries thin CNF prevail on the thick ones. The coronary capillaries are not provided with CNF (Table 2), while the contiguous portions of myocardial tissue appears to be provided with CNF and others nervous structures resembling nerve endings. The coronary veins were provided with few CNF (Table 1). In the great arterial branches, the presence of some elbow shaped masses of AChE positive material very close to the CNF could be observed (Fig. 3,4). QAI performed on a great number of preparations yielded the opportunity to observe relevant regional variations and to highlight the stain's modifications in large and small calibre coronary branches (Table 1).

Therefore, in stretched flat on slides of the external layer (adventitia) or in the serial sections of the left and right coronary arteries as well as of anterior and posterior intra-parenchymal small branches we can observe both AChE and ChAT positive structures that had been identified as cholinergic nerve fibres (CNF) (Tables 3,4). CNF were organised in a plexus localised in outer adventitia or periadventitia. Large arterial branches

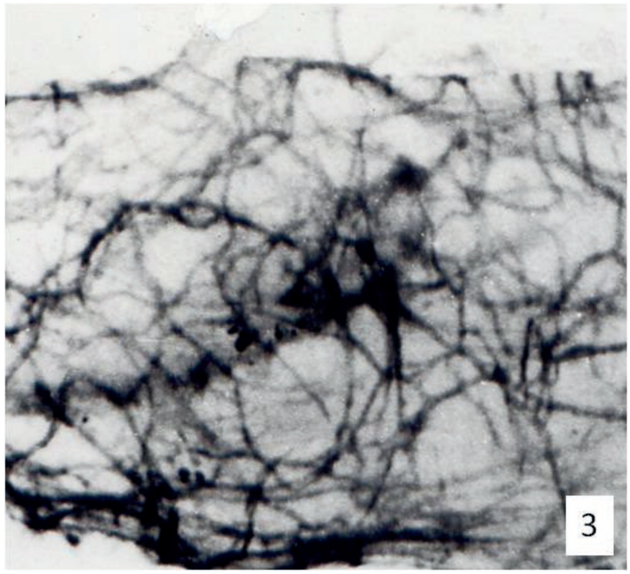

Figure 3. Left coronary artery in a 61-year-old male individual; full-thickness preparation of adventitia. Chat activity after a short time of incubation, periadventitial plexus. Two Chat-positive glomerular structures are located close to cholinergic fibres (Original magnification 450 x calibration bar 10 mm).

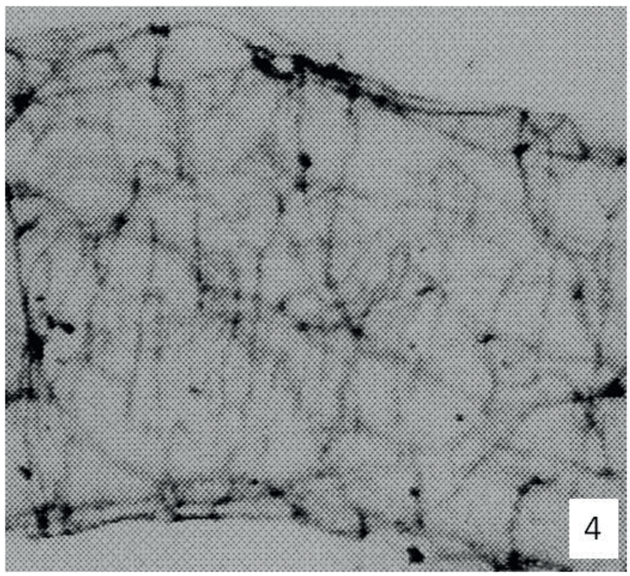

Figure 4. Left coronary artery in the same subject as in Fig. 3 (serial section); full-thickness preparation of adventitia. AChE and ChAT activity, periadventitial plexus. We can observe a strong increase of the staining of AChE and ChAT activity localized in the cholinergic nerve fibres similar as them in surrounding tissues (Original magnification 450 x calibration bar 10 mm).

Table 4. Age-related changes of the ChAT-positive nerve fibres in the human coronary vessels.

Human coronary vessels	Young (n=8)	Old (n=16)
Large extra-parenchymal branches*	29.3 ± 1.8	11.1 ± 1.3**
Small intra-parenchymal branches*	24.2 ± 1.6	7.3 ± 1.6**
Microcirculation (arterioles and capillaries)	0	0
Large and Small veins*	16.8 ± 1.3	5.8 ± 0.7**

* All results are expressed in conventional unit (=CU) ± SEM.
** P = 0.001 older vs younger.

appeared more diffusely innervated than smaller ones. In the larger arteries the periadventitial plexus is formed by thick CNF (from 20 to 30 μm of diameter) and by a few thin CNF (Tables 1,2). In the smaller arteries thin CNF prevail over the thick ones. In the large arterial branches, we observed the presence of some elbow shaped masses of AChE positive material very close to the CNF. QAI on a great number of preparations gave the opportunity to observe important regional variations and to highlight the modifications in old age. About changes related to age it came out that during old age there is a strong reduction of CNF (Table 3).

DISCUSSION

The control by the autonomic nervous system of correct cardiovascular function is influenced by various pathological and physiological factors such as: contractile activity of the myocardium, blood pressure, psycho-physical stress, metabolic diseases; in order to better understand the mechanism through which these factors participate in the onset and progression of cardiovascular diseases, it is essential to know the anatomical characteristics regarding the innervation of human cardiac tissue and above all the role played by aging on cardiac innervations (Mandsager et al. 2015; Millet et al., 2022; Harris et al., 2004).

The heart is innervated by the cardiac plexus, whose formation includes parasympathetic fibres coming from the vagus nerves and orthosympathetic fibres derived from ganglia and trunks of the cervical and thoracic tract of the orthosympathetic chain (Brack et al., 2015; Pather et al., 2003).

Nerve filaments originate from the cardiac plexus which, accompanying the right and left coronary arteries and their branches, are distributed to the heart; some fibres go to the sinoatrial node and the atrioventricular node, others to the atrial and ventricular myocardium and to the wall of the great vessels (Pather et al., 2003).

Our results show that CNF are present in extraparenchymal branches of coronary arteries, while on the contrary CNF are not present in the coronary microcirculation.

Sherf and co-workers (Sherf et al., 1977), studying the ultrastructure of human coronary vessels, described the presence of fibres and nervous endings even in small calibre arteriole and in microcirculation.

In our study, coronary capillaries and microcirculation appear devoid of CNF.

Nevertheless, in coronary blood flow the constriction and the dilatation of microcirculation and/or of the pre-capillary sphincters play an important role. So, the absence of CNF seems to indicate that cholinergic system does not play a role in the coronary microcirculation (Houghton et al., 1998).

More likely, the stained CNF may represent afferent nerve fibres providing information to nervous central system (Shigei et al., 2010).

This hypothesis seems to be supported by the presence of AChE positive glomerular formations in CNF plexus. These formations are similar to cholinergic afferent terminations located in rich baroreceptors areas as aortic arch and the carotid sinus (Norcliffe-Kaufmann et al., 2019). CNF have also been considered as sensory or afferent fibres. These receptors are localized around the large branches of coronary arteries. Brown suggested that these receptors were probably located in the wall or near the large calibre coronary arteries (Norcliffe-Kaufmann et al., 2019).

Acetylcholine is the chemical mediator of postganglionic parasympathetic endings. Since there are at the moment, no techniques able to histochemically demonstrate acetylcholine, cholinergic nervous fibres in peripheral nervous system are pointed out thanks to the stain of enzyme that catabolizing acetylcholine: (acetylcholinesterase-AChE). In origin, the technique used to localize AChE in tissues was proposed in 1953 by Gerebtzoff (Gerebrzoff, 1953). After that were brought many modifications to the original technique to reduce the artefacts (coming from the enzyme diffusion) and to increase the specificity and the intensity of the reaction. Karnowsky and Root (Karnowsky & Root, 1964) introduced the "cholinesterase direct pointing out technique" using thiocholine. This method represents, at the present time, one of the most used techniques to point out AChE at electron microscopy. Nevertheless, histochemical techniques, above mentioned, show and AChE, the 'true cholinesterase', and not specific cholinesterase. To eliminate non-specific cholinesterase, sections must be treated with phosphoric acid esters (DFP, Mipafox, Iso OMPA, etc.); these substances inhibit non-specific cholinesterase without alter the 'true AChE'.

AChE is a rather resistant enzyme. In fact, the sections used to show the histochemistry of this enzyme stand even the fixation in formalin and can be kept in freezer at low temperatures for some months, without reducing the enzymatic activity. Moreover, AChE is resistant enough to the autolytic *post-mortem* phenomenons. However, AChE is not thermoresistant (a temperature higher than 50°C may inactivate AChE). Techniques for cholinesterase localisation using the specific inhibitors, are, at that present, the only techniques able to show cholinergic nervous fibres at optics microscope (Koelle, 1963). In relation to the strong presence of parasympathetic innervation in heart many studies were performed to assess the cholinergic innervation pattern. These studies have highlighted the cholinergic nervous fibres distribution in the heart, in the coronary and pulmonary vessels and in the aorta and caval veins.

It has been demonstrated that CNF are distributed in coronary vessels but in small laboratory animals, coronary vessels have a limited cholinergic innervation. Our findings seem to agree with all these data. Nevertheless, further morphological and functional studies are needed to define the role and function of CNF in the coronary circulation.

In our study, a reduction in CNF was highlighted in elderly subjects compared to young subjects. In the heart of elderly people, changes are found especially at the level of the myocardial tissue and coronary arteries; these changes almost always depend on various pathological factors that occur at an advanced age and which, associated with physiological aging, have repercussions on the correct functioning of the cardiovascular system. Several studies confirm that changes related to aging are caused by the prolongation of the duration of contraction, with a consequent decrease in inotropic responses to catecholamines and cardiac glycosides and an increase in mechanical refractoriness; furthermore, alterations in myocardial relaxation are also highlighted which can be correlated with the prolongation of contractile activity (Bruzzone et al., 2003; Docherty, 2002). In light of these data, it appears clear that the vascular insufficiencies that occur during aging can be a consequence of alterations in cardiac electrical activity, specifically alterations in the duration of the action potential and alterations at the level of the sarcoplasmic reticulum. With age, changes in electrical activity are also evident at the level of the sinoatrial and atrioventricular nodes as well as in the bundle of His and Purkinje fibres. These changes appear to be caused by both anatomical and physiological modifications of the main arteries. Furthermore, aging also appears to be related to a change in the effect and above all in the effectiveness of the drugs used for the treatment of vascular insufficiencies (Bruzzone et al., 2003; Docherty et al., 2002; Njemanze et al., 2016). Therefore, during aging both anatomical and physiological cardiac remodelling occurs with a consequent slowdown of some functions relating to the electrical activity of the heart. It is clear that this process can play a fundamental role in the development and progression of cardiac and vascular insufficiency that develops at an advanced age. The autonomic nervous system also regulates cardiovascular homeostasis through regulation of heart rate, myocardial contraction, and vasoconstriction, contributing largely to such age-related changes (Bruzzone et al., 2003; Docherty, 2002; Njemanze et al., 2016; Francis Stuart et al., 2018).

AUTHORS' CONTRIBUTIONS

ST, AC and VP designed the study. FF, IV, LC, FMG, FC and VS consulted literature and collected data, ST, AC and FF wrote the paper. FMG reviewed and edited the manuscript. All authors read and approved the manuscript.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

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Citation: Cotovanu, A., Bud, M.I., Pleșa, A.V., Cosma-Epure, I., & Cosma, C. (2024). Anatomical insights into double cystic duct: A rare variation observed in cadaveric dissection. *Italian Journal of Anatomy and Embryology*128(2):49-53. https://doi.org/10.36253/ijae-15674

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Anatomical insights into double cystic duct: A rare variation observed in cadaveric dissection

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Abstract. *Introduction.* Rare but significant anatomical differences in the biliary ducts, such as having two ducts, can create challenges in surgeries like laparoscopic cholecystectomy, according to a study that examined a case of double cystic duct found during cadaver dissection and its implications in practice. *Material and method.* In the study, dissections were done on 25 cadavers at the Anatomy Department of the University of Medicine Pharmacy Science and Technology in Târgu Mureş, where a cadaver with a secondary cystic duct anomaly was observed. A series of measurements and histological examinations were conducted to validate the results and juxtapose them with prior literature. *Results.* The secondary cystic duct showed a shape resembling the letter "Y," with separate insertions of the ducts at the common hepatic duct level with the same macroscopic anatomical features. Histological examinations revealed normal biliary epithelium and tubular shaped structure. *Conclusion.* Finding a secondary duct underscores the importance of vigilance during surgery because such variations can raise the chances of bile duct damage during gallbladder cholecystectomy. It is advised to use imaging, before surgery and cholangiography during surgery to manage the risks linked to these abnormalities.

Keywords: double cystic duct, biliary variations, cadaveric dissection, biliary surgery.

INTRODUCTION

Anatomical differences in different organ systems, though common, pose challenges for both medical imaging and surgical procedures. One rare and important anomaly is the presence of a supplementary cystic duct. Potential impact on clinical practice and surgical interventions is present duet to specialized literature reports that emphasize the need to take into consideration that such an anatomical variation might occur. During embryological development, variations may occur due to incomplete fusion or abnormal differentiation of the biliary structures, leading to the formation of a double cystic duct. (1,2)

The cystic duct normally links the gallbladder to the bile duct playing a role in transporting bile. Variations in its structure can increase the risk of complications during biliary surgical interventions and cholecystectomy. A double cystic duct, where there are two ducts coming from the gallbladder, adds another level of complexity to biliary anatomy. Although such anomalies are uncommonly documented they require attention and thorough surgical planning to prevent damage to the ducts and related health issues. In the past, there have been classifications for cystic duct abnormalities, with early literature consisting mostly of sporadic case studies. Flannery and Caster, in 1956, classified these irregularities into three types: the “Y” type, where two cystic ducts join to create one channel; the “H” type, where an additional duct drains separately into the hepatic ducts; and the trabecular type, where an extra duct directly connects with the liver tissue. Recent case studies and anatomical research focus mainly on the clinical significance of these variations. Studies on cadavers offer insights into anatomical differences, providing a controlled environment to explore uncommon abnormalities. Cadaver dissection can add to the valuable data of intraoperative and radiological reports in order to define the role of this very rare variation.

In this study, we describe the cystic duct found in our cadaver specimen, connecting our observations with existing literature to offer a comprehensive understanding of this rare anomaly. By incorporating these insights, we aim to equip clinicians to handle variations, ultimately enhancing surgical outcomes and lowering complication risks.

MATERIALS AND METHOD

The research took place in the Department of Anatomy of The University of Medicine, Pharmacy, Science, and Technology, George Emil Palade” Târgu Mureș utilizing the institution’s collection of cadaveric specimens. Dissection was carried out for academic and learning purposes for the first and second-year general medicine students. A total of 25 cadavers underwent dissection over an approximately six-month period as part of the academic year. Cadavers were chosen without regard to gender, age, or suspected cause of death. All specimens had been preserved following formalin fixation procedures to preserve tissue quality and enable anatomical examination. Anatomists and volunteer-trained medical students conducted the dissections under supervision following established protocols. The dissection began by examining the wall and then carefully exploring the organ systems, within the peritoneal cavity in detail. A closer look was given to the hepatobiliary system because a potential anatomical variation had been found. The gallbladder along with structures like the cystic duct and common bile duct, were revealed along with the surrounding blood vessels. The primary aim of the dissection was to pinpoint landmarks such, as the Calots triangle to ensure an understanding and exposure of the biliary system. When a double cystic duct was identified, detailed morphological measurements were taken, including lengths and diameters of the ducts and insertion with anatomical disposition. The researchers took pictures to record their discoveries. Then, they were compared to anatomical references. They verified the nature of the duct through an examination of the ducts at a macroscopic level. The confirmation process involved analyzing the ducts that were pinpointed and removed. Histological checks were necessary, to confirm the findings. Tissue samples were collected, cut into sections and dyed with Hematoxylin and Eosin (H&E) for assessments. The purpose of this study was to confirm the existence of tissue and identify any alterations, such, as inflammation or scarring, that could be linked to observations during surgical procedures and also to assess the impact of anatomical functionality.

In terms of ethics, considerations were taken into account in the research and educational use of cadaveric specimens, following standards and guidelines that emphasized the importance of respecting the dignity and integrity of these specimens. The research findings were solely aimed at advancing knowledge without any personal interests. This methodological approach offered an in-depth understanding of the variation under investigation, enabling an exploration of its clinical and surgical implications. The collected data underwent analysis. We compared our findings with anatomical and surgical literature to provide fresh insights into the double cystic duct anomaly.

RESULTS

During the dissection of a body, an unusual anatomical variation was discovered. A double cystic duct. This finding was thoroughly documented using a combination of examining the structure, studying the tissues under a microscope, and capturing images to fully understand this abnormality, in the bile duct system. Initially, the gallbladder appeared normal in its place on

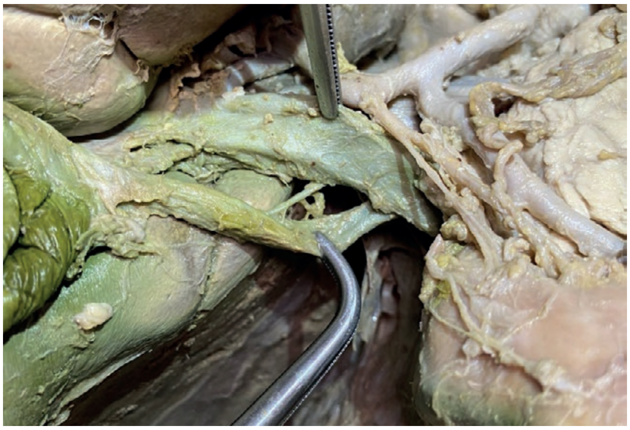

Figure 1. Depicts the initial view of the biliary tree highlighting where the cystic ducts branch off from the gallbladder neck. Using dissection tools showcases how these ducts are positioned („Y" shape pattern).

the liver's surface. However, further exploration uncovered a split from the neck of the gallbladder, indicating two cystic ducts. The main cystic duct followed its path by extending from the gallbladder's neck and connecting with the bile duct at its regular spot in anatomy with no variations present and with the basic anatomical reports present. This primary duct had measurements with a diameter of around 3 mm and a length consistent with what's generally seen in anatomy. The secondary cystic duct deviated from the main and took a distinct course by joining directly with the common hepatic duct in a „Y" shape pattern. This smaller duct had a diameter of 1 mm and the same superficial structure (Figure 1).

Further exploration reveals how the secondary cystic duct connects and merges with the common hepatic duct. This image clearly displays their relationship and proximity to liver tissues. The secondary cystic duct was sectioned, revealing a macroscopically tubular-shaped structure (Figure 2).

After examining the structure tissue samples, the secondary duct was sectioned for analysis under a microscope. The samples were preserved in formalin, cut into sections, and stained with Hematoxylin and Eosin (H&E) to study the cell composition and confirm the presence of epithelium. The secondary cystic duct showed a lining without signs of inflammation, scarring, or abnormal cell growth. The overall structure of the

Figure 2. Sectioned secondary cystic duct with tubular-shaped structure.

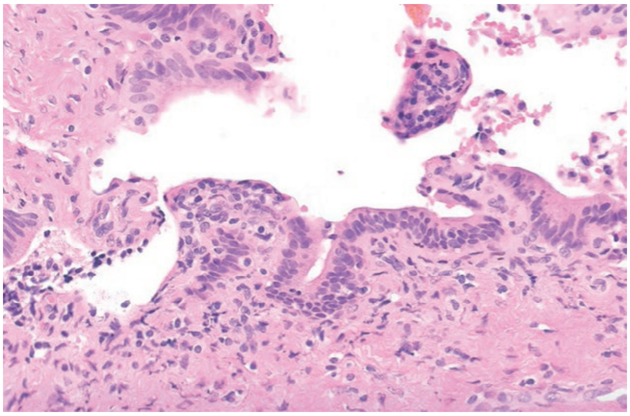

Figure 3. Secondary biliary duct with tubular structure and cell lining.

duct matched that of a duct, confirming its drain role in the anatomical biliary system (Figure 3).

DISCUSSIONS

The discovery of a secondary duct during the dissection of a cadaver offers valuable information about the intricate nature of biliary anatomy. While differences in the tree are frequently encountered, having two cystic ducts is considered an exceptionally uncommon abnormality, with only a limited number of documented instances in the medical literature. Recognizing these variations is crucial for studies and medical procedures in laparoscopic surgery, where unidentified anomalies can result in serious complications (1-4).

In this instance, we noticed a formation that resembles the "Y" shape pattern, with one duct connecting to the common bile duct in its typical position and another duct linking separately above close to the hepatic duct junctions. This arrangement corresponds to the system outlined by Flannery and Caster for classifying duct cases according to their drainage routes. Our case falls under the "Y" type, which is defined by two duct paths merging at points. In line, with findings in existing studies and reports in the literature, it is common for instances of duplicated duct to either converge both into the common bile duct or, independently empty, into the hepatic duct/common hepatic duct (2,5,8). From a clinical viewpoint, the existence of a cystic duct notably heightens the likelihood of bile duct injury, during surgeries like laparoscopic cholecystectomy. Recognizing and addressing aspects of the bile system is crucial to prevent issues like bile leakage or even total severing of bile ducts. These dangers are further complicated by the chance that pre-surgery scans, like Magnetic Resonance Cholangiopancreatography (MRCP), may not consistently spot these deviations. While MRCP has been commonly used to chart the system before surgery, its ability to identify duct structures, like double cystic ducts, is somewhat restricted (2,6,7,11). The double cystic duct was found during a cadaver dissection, in our study, where we could thoroughly investigate the anomaly in a controlled setting. However, in real-life surgeries, this variation may not become evident until the surgeon is operating. In cases of suspected variations during surgery it is strongly advised to use intraoperative cholangiography for live imaging of the biliary tree. Numerous research studies have highlighted the importance of using cholangiography to lower the risk of bile duct injuries in patients with complex or unusual biliary structures (8,5).

The anatomical discovery in this research aligns with what has been observed in cystic duct cases documented in other studies. In a research conducted by Huston and Dakin (2008), they discussed a scenario where a double cystic duct was found during a scheduled cholecystectomy surgery. The abnormality went unnoticed, before the operation; however, during the surgery, cholangiography revealed two duct structures – one connecting to the bile duct and the other leading to the right hepatic duct. (2,4). Anisi et al. (2020), in another study, discovered a cystic duct that proved to be quite troublesome, in the context of laparoscopic surgery, requiring the implementation of sophisticated imaging methods to prevent any potential complications that may arise (5,9-11).

The unique aspect of our study, compared to others, in existing literature, lies in the thorough examination carried out during dissection sessions. While previous studies have mentioned variations like the "H" type – where the additional duct connects to the liver duct separately – our discoveries provide measurements of structures and a detailed analysis of tissue samples that enhance our understanding of this uncommon condition. Encountering a secondary cystic duct presents considerable challenges for surgeons during minimally invasive surgeries due to limited visibility. Even though preoperative imaging tools are useful in cases to identify these anomalies sometimes they might miss detecting rarer variations, which highlights the importance of a thorough assessment during the surgery. It's also crucial to consider the possibility of duct presence, along with other biliary or vascular irregularities, during the procedure (13). At times, such irregularities might be linked to a range of anomalies, within the bile duct system, which could complicate the planning of surgical procedures. For instance, variations in the artery supplying the gallbladder or the existence of multiple bile ducts have been observed alongside cases of double gallbladder. Surgeons need to stay alert for these variations while conducting surgeries as they can impact the technique significantly and heighten the chance of complications, during surgery (14,15).

CONCLUSIONS

During a dissection session, when a double cystic duct is found, it offers information about the intricate nature of biliary anatomy. It highlights the significance of identifying such variations in real-world medical settings. Although uncommon occurrences like these can present difficulties during procedures like laparoscopy, special attention must be given to the use of intraoperative cholangiography and precise dissection to prevent any complications in situations where there is suspicion of a double cystic duct.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Paternostro, F., Lippi, D., Zucchini, E., Nori, J., Galassi, F.M., Nerlich, A.G., & Bianucci, R. (2024). Chronic mastitis or breast cancer in *The Charity* by Francesco Salviati? An educational discussion. *Italian Journal of Anatomy and Embryology* 128(2): 55-60. https://doi.org/10.36253/ijae-15580

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Chronic mastitis or breast cancer in *The Charity* by Francesco Salviati? An educational discussion

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Abstract. In the present communication, the painting *La Carità* (*The Charity*) by Francesco de Rossi, also known as Salviati (1510-1563), is examined from a multidisciplinary perspective, combining the following disciplines: human anatomy, palaeopathology and history of art. In the impossibility to establish a final diagnosis due to the artistic nature of the artwork, the anatomical alterations found on the iconographic representation are discussed in light of two alternative hypotheses, namely chronic mastitis or breast cancer. Once more the intersection between medicine and art can be found to be of excellent use both for increasing the ancient record of diseases and in an educational effort to train the eye of future physicians.

Keywords: art and medicine, breast cancer, chronic mastitis, human anatom, palaeopathology.

INTRODUCTION

Women's breasts have always had a powerful impact on collective imagination due to its double role linked to maternity and femininity (Pluchinotta, 1985; Bianucci & Nerlich, 2022). Over the past decades, biomedical scholars have intensively focused their attention on the shape and the morphology of breasts in works of art with the scope of identifying the antiquity of senological diseases. This led scholars to propose several cases of breast pathologic conditions in statues, paintings and engravings (Grau et al. 2001, Grau and Estrach, 2008, Vaidya, 2007; Sosa, 2007; Lazzeri et al., 2016; Bianucci et al., 2018; Perciaccante et al., 2019, Nerlich et al., 2022). Often these initial diagnoses were challenged. This was, for instance, the case of Margherita Luti, known as "*La Fornarina*" painted by Raphael in 1520. While Espinell (2002) claimed that "*La Fornarina presents signs* (in the left breast*) that are not only diagnostic but also allow staging of the malignancy*", Gross (2004) showed that Margherita Luti lived at least one to two years after having been depicted by Raphael. She, then, entered a house for solitary women. An exceptional long-term survival of a late-stage cancer would have been inconsistent with an untreated pathology. Similarly, Gross challenged other two cases of proposed left breast cancers in Rembrandt's *"Bathsheba"* (1654) [the model, Hendrickje Stoeffels, was Rembrandt's wife; she lived 9 years after the completing of the canvas and died in 1663] (Braithwaite and Schugg, 1983) and in Rubens's "*The Three Graces*" (1630-1635). A left breast cancer of the third Grace, on the right of the canvas, was diagnosed by Grau and co-workers in 2001. However, the model with the apparent pathological evidence of malignancy was Hélène Fourment, Rubens's wife. She died in 1673 thus surviving the attributed breast cancer for 30 years (Gross, 2004). More recently, Lefrère and co-workers (2024) pointed out several pitfalls in the iconodiagnosis of abnormal senological features in examining Dürer's *Eve* (1507), the Le Nain brothers' *Venus in Vulcan's forge* (1641) and James Barry's *Jupiter and Juno on Mount Ida* (1790-1799). They also underlined the need to perform diagnosis of exclusion in particular when pathographical data are lacking.

Although senological abnormalities in the artistic canon may suggest breast tumours, pathological tumour mimickers have to be considered as differential diagnoses. As a matter of fact, various types of inflammation (lactational mastitis, chronic non-puerperal mastitis, including tuberculous mastitis), adenofibromas, cysts and other benign lesions may have the same initial presentation (Lefrère et al., 2024).

Here we describe an abnormal morphological presentation of the right breast in the painting *La Carità* (*The Charity*) by Francesco de Rossi, also known as Salviati (1510-1563) (Figure 1).

METHODOLOGY

The painting is examined by adopting the principles of iconodiagnosis as applied to the palaeopathological study of disease and the training of future medical generations (Charlier et al., 2023; Galassi et al. 2023; Rühli et al. 2016).

Figure 1. *La Carità* (Inv. 1890/ 2157), ca. 1545, oil on panel, 156 x 122 cm, is held by the Uffizi Gallery (Florence). Reproduced with permission.

RESULTS

In the visual arts, this allegory of a Theological Virtue is sometimes represented as a woman with bare breasts, symbol of the unconditioned love that she gives to others. This type of artistic representation reminds of the exemplary tale of the *Caritas Romana* (which was rooted in the Greek one), where a young woman, Pero, secretly breastfeeds her incarcerated father, Cimon, who had been sentenced to death by starvation. The Latin tale is contained in the book *Factorum et dictorum memorabilium, libri ix* ("Nine Books of Memorable Deeds and Sayings", ca. 31 CE) (https://www.thelatinlibrary.com/valmax9.html) by the Roman historian and moralist Valerius Maximus (1st century CE). This tale had a huge impact and popularity in the Late Middle Ages and the Renaissance.

According to traditional iconography, Salviati's *Charity,* a young woman, dressed in red, shows her right

Figure 2. a: Statue of the *Venus Genetrix* type held by the Ducal Palace in Mantua. Originally owned the Roman antiquarian Giovanni Ciampolini at the beginning of the 16th century, the statue was brought to Mantua by Giulio Romano in 1526 (Haskell & Penny 1984, 495 n° 91, 23-25; Viljoen 2001, 395; Gorrini 2008, 195-202); b: According to the results of the last restoration (Cornini 2020, pp. 277-280), the oil painted image of *Iustitia* was depicted by Raphael himself on the walls of the Room of Constantine in the Vatican Museums.

breast in the pose of breastfeeding. In this case, she does not feed either a father or a mother, but she is surrounded by three children.

The shape of the Charity's visible breast imitates similar anatomical details of some antique Venuses (Figure 2a) and of Raphael's fresco, *Justice* (*Iustitia*) in the Room of Constantine (Vatican Museums), recently restored and definitively attributed to the master (Figure 2b) (Mendelsohn 2001, pp. 108-130, 134-148; Haskell & Penny 1984, p. 495 n° 91, 23-25; Viljoen 2001, p. 395; Gorrini 2008, pp. 195-202; Cornini 2020, pp. 277-280).

On higher magnification and detailed inspection of the painting a well- formed female breast presents with precise morphology. The nipple, which is neither retracted nor fissured, appears slightly erected and the orifice contains minimal traces of a whitish fluid, such as a small milk droplet. In contrast, the adjacent areola presents in its medial inferior quadrant a tiny defect such as seen in a small fistula. This seems associated with an ovoid lump that slightly bulges the skin, but there is neither ulceration nor any discoloration or reddening of the skin (no evidence for "peau-d'orange"). On further inspection, in direction to the axilla, there exists a second lump with smooth surface and without any coloration or defect of the overlying skin. This is positioned just medially to the usual small fat pad that is seen in numerous regular axillae (Figure 3).

A comparison between photographs taken before and after the restoration of the painting shows no differences in the depiction of the breast. Whether an underdrawing was found in pre-restoration investigations is not known, since the Archivio Restauri of the Uffizi is

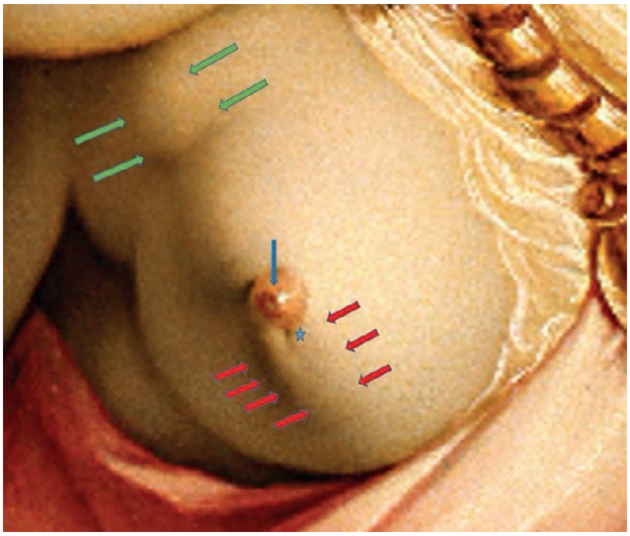

Figure 3. Close up of The Charity's right breast shows the details of the painting: While the nipple seems indurated (blue arrow), there is a small fistula at the areolar margin (asterisk) which is adjacent to a lump of the lower inferior quadrant (red arrows). Additionally, there is a second lump in direction to the axilla (green arrows) superior to the usual axillary fat pad.

temporarily inaccessible (https://www.wga.hu/art/s/salviati/1/charity.jpg; https://www.uffizi.it/opere/carita; https://www.uffizi.it/pagine/ufficio-restauri).

DISCUSSION

The interpretation of the aforementioned observations is particularly difficult and requires a balanced differential diagnosis – as far as possible in iconodiagnostic settings. These include, beyond biomedical evaluation, the evaluation of artistic influences, style and historic settings. When these rules are applied to the canvas under discussion, we observe that the painting provides an extremely realistic image of a female breast in a young woman with distinct pathological alterations. Therefore, the mere representation of an artistic canon can be excluded. Moreover, there is also no apparent evidence for any modification of the canvas, such as durinag restoration processes. Accordingly, the image can be attributed to distinct pathological changes. These comprise an areolar fistula, a circumscribed lump of the medial inferior quadrant and a "swelling" in direction to the axilla. There is no retraction of the nipple, no discoloration of the skin and no ulceration. The two major differential diagnoses comprise breast cancer and breast inflammation.

When taking mastitis into account we have to regard firsthand arguments of a presumed lactational status of the young woman. In this case, an inflammation (mastitis) may be taken into account as there seems to be an areolar fistula. However, acute (puerperal) mastitis usually causes a reddening of the skin, which is absent in the image.

The next possible differential diagnosis may be chronic (non-puerperal) mastitis. This may include the fistula and both lumps – taking reactive lymph node swelling as possible reason for the axillary lump into account. However, non-puerperal mastitis is usually seen in older women; this may also include tuberculous mastitis which may have very long-standing progressive clinical courses (Nicholson et al., 2009).

So finally, the most important differential diagnosis must be breast cancer which usually presents with a breast lump, as shown in the canvas. Furthermore, the axillary lump may represent a putative axillary lymph node metastasis. A fistula is not a typical feature of breast cancer, which in turn, would usually present with either an ulceration and/or reddening and infiltration of the skin (termed "peau-d'orange" in the so-called "inflammatory" variant of breast cancer). Neither of these typical features is seen, and, furthermore, breast cancer is very rare in young women. A study of untreated historical breast cancer cases (covering the years 1804-1933) revealed only 0.8% of cases in the age group between 20 and 30 years (Bloom et al., 1962), which is the most likely age group of the *Charity*.

Finally, as a special type of breast cancer, Paget's disease of the breast has to be discussed. This rare form of breast cancer affects the nipple and/or the areola; this type of cancer is prevalent in women aged over 50. This presentation results from a non-invasive infiltration of nipple/ areola from a mostly non-invasive "in-situ" breast carcinoma (Hamzah et al., 2019). This type of breast cancer can be ruled out in the canvas since the nipple and the areola do not show any of the aforementioned signs.

CONCLUSIONS

In summary, Salviati's painting represents an impressive example of the challenges scholars are faced with when dealing with breast pathology. None of the criteria of the aforementioned differential diagnoses fits to 100% with the image; however, taking young individual age and the pathological feature into account, a persistent chronical (puerperal) mastitis may be the most likely diagnosis, although an early form of an aggressive breast cancer (in an unusually young woman) cannot be fully excluded. A conclusive diagnosis cannot be reached since the identity of the model is unknown and, therefore, pathographic data are absent (Charlier et al., 2023; Rühli et al., 2016).

In 2018, we identified two 16th century probable cases of breast cancer depicted by Maso da San Friano (*The Allegory of Fortitude* painted in 1560-1562, Galleria dell'Accademia, Florence, Italy) and Rodolfo del Ghirlandaio (*The Night* painted in 1555-1565, Galleria Colonna, Rome) (Bianucci et al., 2018).

While later studies confirmed the diagnosis of nipple breast cancer in *The Night* by Rodolfo del Ghirlandaio (1483-1561), the case of *The Allegory of Fortitude* has been challenged (Nelson, 2021).

Actually, an examination of the *Allegory of Fortitude* after its restoration in 2003, which removed yellowed patinas and later alterations, shows visible *pentimenti* on the right side of the torso, retouchings which turned transparent over the years, thus calling the previous hypothesis into doubt (Falciani & Natali, 2017, pp. 266-267). It should be noted that Maso da San Friano was influenced not only by Michelangelo (as was Salviati, who probably recalled the breasts of Buonarroti's *Night* while painting the *Charity*), but also by Pontormo and Rosso Fiorentino, Mannerist painters whose female figures often have asymmetrical breasts - see Pontormo's *Leda and the Swan,* Rosso Fiorentino's *Death of Cleopatra* (Cheney 1991; https://it.m.wikipedia.org/wiki/File:Jacopo_Pontormo_-_Leda_and_the_Swan_-_WGA18073.jpg; https://it.wikipedia.org/wiki/Morte_di_Cleopatra_%28Rosso_Fiorentino%29).

Here we add further knowledge to the accuracy of 16th century representations that have been executed so perfectly, that even breast cancer and/or its mimickers may be identified. This paper highlights the existence of an intimate link between figurative arts and biomedical sciences during Renaissance; this was the period when the study of the human body was a multifold experience that involved both the artist and the man of science who sometimes were the same person as in the case of Leonardo da Vinci.

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IJAE
ITALIAN JOURNAL OF
ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Saccheri, P., Crivellato, E., & Travan, L. (2024). A forgotten debate in the history of medicine: vascular anastomoses, from open functional relationships to tight structural connections. *Italian Journal of Anatomy and Embryology* 128(2): 61-73. https://doi.org/10.36253/ijae-15607

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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A forgotten debate in the history of medicine: vascular anastomoses, from open functional relationships to tight structural connections

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Abstract. According to current anatomical nomenclature, vascular anastomoses are direct connections between blood vessels. The Greek word '*anastomōsis*' means 'opening' and conveys the idea of 'patency' rather than 'connection'. *Anastomōsis*, and the related term *sunanastomōsis*, originally referred to a mere approximation of the mouths of adjacent blood vessels – especially arterioles and venules – facing each other at their terminal ends. These vessels were not strictly connected and did not form anatomical units in the proper sense. Over time, however, the term *anastomōsis* underwent a significant semantic change and now denotes a closed, structured anatomical connection. How and when did this semantic shift occur? The aim of the present study was to evaluate the concept of *anastomōsis* in different historical periods from Antiquity to the Seventeenth century and to investigate how this entity gradually lost its original meaning of mere functional opening to acquire the notion of true anatomical structure.

Keywords: modern anastomosis, anatomy, physiology, history.

INTRODUCTION

Vascular anastomoses are direct connections between blood vessels (Standring, 2005). The term implies a state of structural, physical continuity between the vascular components involved. Anastomoses can be divided into arteriovenous anastomoses, anastomoses between arteries and anastomoses between veins. Arteriovenous anastomoses occur between smaller arteries and veins, with no capillary section between them. Connecting vessels may be straight or tortuous, and often have a thick muscular tunic. They are widespread in the body and are most common in the skin of the nose, lips, ears, the nail-beds of the fingers and toes, nasal and digestive mucosa, and erectile tissue. Arteries can also be connected by an anastomosis, allowing to supply the area of the other. An end-to-end anastomosis occurs when two arteries communicate directly with each other. An anastomosis by convergence occurs when two arteries converge and merge. Transverse anastomoses are also described. Anastomoses also occur between veins. They form connecting channels or lead to venous plexuses. Anastomoses may be macroscopic or microscopic. In any case, they denote direct connections between distinct blood vessels.

Vascular anastomoses have played a crucial role in the history of medicine and science. In Antiquity, as well as in Middle Ages and the Renaissance, according to Galen's teachings, they were assumed to be critical sites for the exchange of material, namely refined blood and *pneuma*, between small, invisible arterioles and venules in the organs of the body. During the sixteenth and seventeenth centuries, anastomoses were considered as the main route for blood passage from the venous to the arterial compartment. In a circulatory perspective, Harvey first conceived of them as one of the two routes by which blood would pass from the veins to the arteries.

The term 'anastomosis' is derived from the Greek word '*anastomōsis*' (ἀναστόμωσις). It means 'opening' and conveys the idea of 'patency' rather than 'connection'. The cognate verb '*anastomoō*' (ἀναστομόω) means 'to provide with a mouth', 'to open', also 'to be opened, dilated'. *Anastomōsis* and the related term *sunanastomōsis* (συναναστόμωσις) were used by the great anatomist and physician Erasistratus of Iulis on Ceos, who lived in the earlier half of the third century BC. According to von Staden, "*Sunanastomōsis* is apparently one of Erasistratus' many neologisms, formed with a characteristically Hellenistic double prepositional prefix" (von Staden, 1975). Aristotle had already used the formula "τῶν φλεβίων ἀναστομωθέντων", to refer the tiny blood vessels that opened on the surface of the skin as a result of intense heating of the body (Aristotle, *De partibus animalium*, III.5, 668b5). Modern scholarship has interpreted ancient *anastomōseis* as closed, continuous structures. They have been equated with capillaries, which are microscopic endothelial tubes directly linking arterioles to venules, or with connecting vascular channels. Harris stated that Erasistratus "seems to have invented rather than discovered the capillary system", and Furley compared the *anastomōseis* to "invisibly small channels" which connected the arteries to the veins at the extremities of both (Harris, 1973; Furley, Wilkie, 1984). This misleading view is no longer tenable. Recently, David Leith has provided textual evidence for a new interpretation of the *anastomōsis* construction in the teaching of Erasistratus. According to Leith's analysis, Erasistratean *anastomōseis* (Galen, *De venae sectione adversus Erasistratum*, 3, K.11.153 = fr. 198 Garofalo, 1988), contrary to what the term would imply according to modern anatomical nomenclature, do not denote the connection and physical continuity between invisible arterial and venous structures but merely involve a proximity of the terminal openings (ἔσχατα στόματα) placed at the extremities (πέρατα) of adjacent arterioles and venules He argues that this minute openings or mouths placed at the extremities of the invisible arteries and veins of the triplokia "are not joined together at their ends, but lie alongside one another in every part of the body" (Leith, 2015). There is thus a small interspace, or *hiatus*, between the adjacent vessels. Erasistratus conceived of the vascular system as consisting of two distinct and separate branches: the *pneuma*-transporting arteries and the blood-conveying veins (ps.-Aristotele, *De spiritu*, 5, 483b28-31, 484a2-3; Lewis, Gregoric, 2015). There were no connexions between the two districts. The *pneuma* flows exclusively in the arterial compartment and the blood in the venous. The two substances never meet and mix under physiological conditions. Arteries and veins originate in the left and right ventricles, respectively. Both kinds of vessels divide and branch again and again to their way to the various organs of the body, gradually diminishing in size and eventually becoming invisible. At their outermost periphery, the subsensible arterioles and venules, perceptible only to reason, lie side by side and next to the invisible nerves, intertwined and embedded in ubiquitous elemental structures called the *triplokia tōn angeiōn* (τριπλοκία τῶν ἀγγείων), the 'triple web' of elastic vessels. The microscopic artery and vein of the *triplokia* terminate with tiny orifices or mouths lying alongside one another (παρακειμένων ἀλλήλοις τῶν στομάτων). Normally, these *stomata* remain closed and the blood is held in the small veins (Lonie, 1964; Harris, 1973; Garofalo, 1988; Vallance, 1990; Vegetti, 1998). When *pneuma* escapes from the arterial district or a plethora condition occurs in the venous system, there is an 'opening' of the *stomata* (*anastomōsis*) at the venous terminations and blood runs rapidly into the arterial conduits. Thus, an 'open' pathway develops between venules and arterioles, which necessarily causes disease. In Erasistratus' theory of the *triplokia*, terminal vessels are not directly joined. They do not form a 'closed', structured anatomical connection. Such a spatial arrangement is expected to imply functional significance. In Erasistratus' doctrine of the *anastomōseis* there seems to be no place for any physiological function, since in the normal state there are no *anastomōseis* at all. In the ordinary condition, the terminal *stoma* of the imperceptible vein of the *triplokia* remains closed and the nourishing blood is poured out in the surrounding *parenchuma* – that is the flesh of the organ – at the side of the vein (κατὰ τὰ πλάγια). Thus, the *anastomōsis* appeared to play a role only in pathology and was closely connected with it. So, we assume that the Erasistratean *anastomōsis* is to be conceptualized not so much as an anatomical structure but as a functional condition.

The term *anastomōsis* then did not initially imply physical continuity between the vessels involved. It was not intended to denote a connecting vascular channel. How and when did this semantic shift occur? In what way did the Erasistratean *anastomōseis* become the modern anastomoses? The aim of this paper was to evaluate the meaning of the concept of *anastomōsis* in different historical periods from Antiquity to the Seventeenth century and to explore how this entity gradually lost its sense of mere functional opening to acquire the notion of true anatomical structure.

THE ANTIQUITY

Around the time of Erasistratus, about the first half of the third century BCE, we have the interesting testimony of the pseudo-Aristotelian author of *De spiritu*. He asserts that the air-ducts (*artēriai*) and the nutriment-ducts (*phlebes*), which extend along the ribs "are connected by contact" (τὰς δὲ φλέβας καὶ τὰς ἀρτηρίας συνάπτειν εἰς ἀλλήλας) and exchange substances, because "the *pneuma* needs the liquid and the liquid needs the *pneuma*" (Ps.-Galen, *Introductio sive medicus*, 9, K.14.697-698; Manetti, 2011; Galen, *De usu partium*, VII.8, K.3.538; *De anatomicis administrationibus*, II.11, K.2.337; *De facultatibus naturalibus*, II.6, K.2.105; *De venae sectione adversus Erasistratum*, 3, K.11.153 = frs. 86, 87, 88, 90, 148, 198 Garofalo; Leith, 2015). In this case, there seems to be a real contact (συνάπτειν) and not a mere proximity between the vessels. The last sentence probably implies a general physiological view of the author and suggests that these connections may be present throughout the human body. Remarkably, the author of this short treatise does not use the word *anastomōsis*, or related terms, to refer to this type of vascular connection. This would suggest that what was called *anastomōsis* did not mean a tight junction by contact of the vessels, but a loose proximity of their endpoints. This seems to be important testimony, because given the general lack of ancient textual accounts, it appears the only one that departs from the teaching of Erasistratus and clearly implies the notion of a close connection between opposing vessels.

In the second century AD, Galen derives the idea of *anastomōseis* from Erasistratus. In his doctrinal system, however, these vascular arrangements acquire new functional roles. They perform a crucial task even under normal conditions, exchanging small quantities of *pneuma* and blood of the finest and purest quality through the orifices of the thinnest, invisible arterioles and venules. In this way, a peripheral relation will be established between arteries and veins, symmetrical to the central one given by the pores in the *septum interventriculare*, and the advantages derived from respiration and pulse would be beneficial not only to the heart and arteries, but also to the veins.

The idea of *anastomōseis* or *sunanastomōseis* – Galen seems to use both terms without any significant difference – came to Galen from the empirical observation that animals slaughtered by severing a major arterial vessel bled out quickly and completely. He interpreted this observation by postulating the existence of *anastomōseis*, or 'openings', between the extremities of the vessels (τὸ πέρας ἀνεστομωμένων τῶν ἀγγείων), veins and arteries (Galen, *De facultatibus naturalibus*, III.15, K.2.207; *De usu pulsuum*, 5, K.5.165; *De methodo medendi*, V.2, K.10.311; Furley, Wilkie, 1984). Blood flows from the venous quarter into the arterial compartment through "small orifices" found at the ends of blood vessels. In *De methodo medendi* (V.2, K.10.311), Galen mentions τὸ στόμιον, "the tiny mouth" of the blood vessel participating in anastomōsis, and the small size of the vessels involved, "δι' ἀναστόμωσιν ἀγγείων μικρῶν". In *De usu partium* (VI.10, K.3.455-456) the Pergamene asserts that arteries and veins "open their orifices" (συνανεστόμωνται) in every part of the body, and exchange blood and pneuma "precisely through certain invisible and narrow passages" (διά τινων ἀοράτων τε καὶ στενῶν ἀκριβῶς ὁδῶν). Galen insists on the concept of the invisibility of these passages (ἀόρατα), which are so small that they "escape sight" and become "subsensible" (Galen, *De usu partium*, VI.21, K.3.510; *De usu pulsuum*, 5, K.5.165; Furley, Wilkie, 1984).

How was the structure of these *anastomōseis* conceived by Galen's doctrine? He leaves the matter somewhat unsettled. This problem is called into question in *De locis affectis* (V.7, K.8.351-352), where Galen describes the intrahepatic arrangement of the vascular branches of the portal vein and the hollow vein (the vena porta and vena cava of the modern anatomical nomenclature). He says that both vessels terminate with very small endings, which arrive at the same places in the liver flesh and form imperceptible *sunanastomōseis*. Indeed – Galen adds – no one doubts that there are invisible passages between these vessels, through which the nourishment conveyed by the portal vein is received by the branches of the hollow vein, being taken up by the very extremities of the latter. Thus the intralobular sinusoidal capillary system (Standring, 2005) was unknown to ancient Greek physicians and conceptualized as consisting of anastomōseis. This point is closely related to Galen's concept of sanguification, that is, the production of venous blood suitable for nourishing the body. As for his related ideas about the movement of the nutritive blood in the liver, it is sometimes difficult to reconcile the assertions he makes in different places and presents in different contexts for different purposes (Galen, *De usu partium*, IV.3-5 and 12, K.3.269-273 and 298-300). In Galen's physiology, the flesh of the liver is the principal instrument of hemopoiesis and the source of the veins. Both the hollow vein and the portal vein originate in this viscus. The nature of the liver flesh is very similar to coagulated blood. Thus, the juice of the *chyle* absorbed from the stomach is transformed by the *parenchuma* of the organ and gradually converted into mature blood. Whether this process takes place within the hepatic vessels or whether it requires the spread of the *chyle* juice through the liver flesh and its re-entry as mature blood into the thin vessels connected with the hollow vein, Galen did not state openly. The second hypothesis, however, seems to have been favoured by the Pergamene. In *De usu partium* he states that "the veins of the convex part [of the liver] are not connected (οὐ συνάπτονται) with those of the concave part". If their extremities are not joined together there must necessarily be an interruption between their *stomata*. The structure of liver *anastomōseis* is further discussed by Galen in *De facultatibus naturalibus* (II.5, K.2.93-94 = fr. 146 Garofalo). Here, the Pergamene reports a passage from the first book of the *General Principles* of Erasistratus, in which the Alexandrian physiologist speaks of the structural architecture of the common space into which the subdivisions of vena porta conduct the unpurified blood, and from which the bile ducts take the bile, and the branches of vena cava receive the purified blood. Says Erasistratus:

As there are two kinds of vessels opening at the same place, one kind leading to the gall-bladder and the other to the vena cava, it follows that of the nutriment conveyed upwards from the alimentary canal, that part which fits both kinds of *stomata* is received into both kinds of vessels, a part being conveyed into the gall-bladder and the rest passing over into the vena cava. (Galen, *De usu partium*, IV.13, K.3.301)

According to Galen's commentary, it is difficult to say what we are to understand by the words "opening at the same place" (εἰς τὸ αὐτὸ δ' ἀνεστομωμένων*)* which appear at the beginning of the passage. Galen asks whether it is meant that there is either a 'junction' (συνάπτειν) between the end of vena porta and two other vessel ends (biliary and vena cava), or we must suppose that there is, as it were, a common space for all three vessels, which is filled by portal vein, and empties into both the biliary duct and the branches of vena cava. Galen seems to support the latter hypothesis. *Anastomōseis* are openings of the vascular terminal *stomata*. They do not condition a connection by close contact or even continuity of the parts, but the mere proximity of the paired vascular orifices. Galen also uses the cognate verb ἀναστομόω to refer for instance to the dilation of the external uterine orifice and vaginal canal, in a context where the idea of a physical or structural connection is not present (Galen, *De facultatibus naturalibus*, III.3, K.2.150). The verb ἀναστομόω is attested with the same meaning already in the Corpus Hippocraticum (*De natura muliebri* 37, L.7.380.13; *De mulierum affectibus I* 11, L.8.44.1, 13, L.8.50.15; 52.5, 84, L.8.210.4, 89, L.8.214.9; *De mulierum affectibus II* 110, L.8.238.6, 115, L.8.248.23, 116, L.8.252.5; *De sterilitate mulierum* 221, 8.426.9,11,14).

The way in which the intrahepatic veins are connected was described in Antiquity in a mode that suggests the idea of an open type of vascular interaction. Unfortunately, there is much missing in the written medical record between the extant citations from the work of Erasistratus and the work of Galen. We do know that Aretaeus of Cappadocia in the I-II century AD mentions the thinnest, invisible endings of the portal vein, whose orifices in the liver parenchyma "lie together face-to-face" (κατὰ τὸ στόμα ξυγκέονται) with the *stomata* of the inconspicuous, terminal segments of the vena cava (Aretaeus, *De causis et signis acutorum morborum*, II.8). These structures probably correspond to the *anastomōseis* of Galen. As early as the fourth century AD, Oribasius (c.325-400) gives a simplified account of the interaction between the intrahepatic branches of vena porta and vena cava, which seems to depend on that of Galen. However, he does not mention the presence of *anastomōseis* and simply states that the nourishment transported from the digestive tract through the portal vein to the liver is converted into blood and "received back" (αὖθις … μεταλαμβάνεται) by the vein located at the convexity of the organ, called the vena cava (Oribasius, XXV.60). This linguistic formula actually suggests the presence of an open intrahepatic movement of blood. The bishop Nemesius of Emesa lives about the same time as Oribasius (c.340-400). He possesses a profound knowledge of the works of Galen and of ancient Greek medicine. Nemesius makes no mention of intrahepatic *anastomōseis* between the endings of vena porta and vena cava but insists on the close resemblance of the liver parenchyma to coagulated blood and on the property of the hepatic flesh to convert intestinal juice into mature blood (Oribasiusius, *De natura hominis*, vol. XL, ch. 23, col. 696, 25-26). These ideas support the notion that the crude intestinal fluid is poured out of the extremities of vena porta into the liver substance and re-enters the endings of vena cava as mature blood.

Still in the Byzantine Empire, the court physician Theophilus Protospatharius (VII century) writes that the terminal branches of vena porta and vena cava "converge" (συμβάλλουσιν ἀλλήλαις) in the liver parenchyma "through their tiny extremities" (κατὰ τὰς περατώσεις αὐτῶν στενοτάτος οὔσας)(Theophilus Protospatharius, *De corporis humani fabrica*, II.13). "Through these terminals – Theophilus explains – the vena cava receives (μεταλαμβάνει) the purified and concocted blood from vena porta". Since blood preparation – Theophilus clarifies in line of Galen's doctrine – involves a complex process of improvement and refinement of the attracted juice, which includes the elimination of toxic products through the bile ducts, his account supports the view of an open relationship between the intrahepatic venous terminals.

THE MIDDLE AGES

In the Middle-Ages, Greek medical doctrine was transferred to the Islamic world. Accordingly, the notion of the structure of the *anastomōseis* shifted from a Galenic context to a more composite cultural setting. In Islamic medical teaching, the doctrines of Hippocrates and Aristotle were held in high esteem and given equal status to the canon of Galen. This point was also taken up in the theme of the present study. Thus, the spatial organisation of the intrahepatic *anastomōseis* became the subject of different views. In the *Kitāb al-Mansūrī* or *Liber ad Almansorem*, al-Rāzī (died c.925) describes the subdivision of the portal vein into a myriad of tiny vessels distributed throughout the liver parenchyma (al-Rāzī, 1497). Remarkably, these channels look like thin hairs and, in the Latin translation of the Arabic text, "are connected" (*jiunguntur*) to the roots of vena cava, which also have a hair-like appearance. In this way, blood nourishment "moves" (*transeat*) from vena porta to vena cava. Al-Rāzī does not mention the presence of *anastomōseis* and advocates for the first time the concept of a continuous hepatic venous network. He may have been influenced by ancient Hippocratic writings, some of which insist on the idea of a structural continuity in the constitution of the human body, likened to the geometric figure of the circle (*De locis in homine I*, L.6.276.2; *De victu I*.19, L.6.492.24; *De alimento IX,* L.9.102.1; Pormann, Savage-Smith, 2010). On the other hand, Ibn Sinā (d. 1037) in the *Canon of Medicine* seems to support the Galenic doctrine of an open circulation. He states that the small intrahepatic subdivisions of vena porta "run to meet the openings" (*occurrentibus orificijs*) of the roots of vena cava (Ibn Sinā, 1493)(Liber Canonis, book I, fen 1, doctrina 4, ch. 7). Ibn Sinā equates *anastomōseis* with 'constrictions'(*co angustationes*) and maintains that the *chyle* enter them only when mixed with aqueous fluid, which serves as an efficient vehicle. This is the strict doctrine of Galen, the term '*coangustationes*' translating the Greek 'φλέβας στενάς'(*De usu partium*, IV.5, K.3.273). Thus, Ibn Sinā apparently considers the intrahepatic *anastomōseis* as the convergence of the orifices of adjacent blood vessels facing each other at their terminal ends. This concept will play an important role in further discussion about the structure of *anastomōseis* because of the great influence Ibn Sinā exerted on the development of scientific and medical culture in the Latin world of Middle-Ages and Renaissance. The *Canon of Medicine* was destined to become one of the most important books in the academic education of physicians for centuries (Siraisi, 1987). In the Islamic cultural sphere, we find two other relevant figures: Al-Majūsī and Ibn Rushd. In the *Kitāb al-Malīkī* or *Liber regius*, Al-Majūsī (*fl.* c. 983) simply states that the subdivisions of the vena cava "attract the juice of the nourishment" (*attrahunt succositatem cibi*) from the branches of vena porta (Al-Majūsī, 1492). In the *Book of General Principles* or *Colliget*, Ibn Rushd (d. 1198) writes that hairy subdivisions of the portal conduit "meet" (*obviatur*) the hairy branches of vena cava (Ibn Rushd, 1482). The *chyle* nourishment is pooled in the branches of the portal channel, concocted in the hepatic *venae capillares*, and then leaves the liver through the roots of the vena cava. We are dealing here with a closed conception of hepatic movement. The *chyle* does not have to be poured out into the liver flesh to be converted into blood but remains in the very thin intrahepatic vessels. Ibn Rushd was a highly praised scholar of Aristotle. One of the most distinctive teachings in Aristotle's biological canon was that blood has its natural place in the vessels and always flows in them, otherwise it putrefies (*Historia animalium*, III 2, 511b1; *De partibus animalium*, III 4, 665b13). Thus, Arab physicians and scholars have taken a variety of positions on the question of *anastomōsis* structure, possibly reflecting the different reception of ancient doctrines.

In the Western world, with the establishment of the early *studia* of academic medicine, we witness the rise and development of a lively debate on the nature of *anastomōseis*. Towards the end of the thirteenth and beginning of the fourteenth century, Petrus Turisanus (died c.1320), the pupil of Taddeo Alderotti, the author of the most important and influential fourteenth-century commentary on Galen's Tegni (technē iatrikē, Ars medica) and one of the most influential representatives of Scholastic medicine, wrote that arteries and veins exchange blood and spirit through "aperture of invisible mouths" (*apertione orificiorum occultorum*) placed throughout the body. In this way, the arteries convey the spirit to the veins, and equally receive the blood through "strict and invisible passages" (*ex vijs strictis, latentibus visum*)(Turisanus, 1557). In these words Turisanus seems to have correctly interpreted the meaning of Galen's *anastomōseis* or *sunanastomōseis*, but when we move on to examine his notion of intrahepatic *sunanastomōseis* we find that he supports the concept of a close structural continuity in the hepatic vein architecture. He writes that the *vena chylis* (vena cava) reaches the convexity of the liver from the right cavity of the heart and then branches into countless divisions throughout the liver substance (Turisanus, 1557). At the concavity of the organ these veins unite into a single vessel called the vena porta. The organisation of the hepatic veins thus appears to be that of an uninterrupted system of conduits. Turisanus does not mention liver *sunanastomōseis* at all.

On the other hand, we find Peter of Abano (c.1257-1315), the eminent physician, philosopher, astronomer and astrologist of the same age, writing at the beginning of the fourteenth century that:

When the *chyle*, attracted by meseraic veins, reaches the thinnest branches of vena porta, it runs to meet (*occurrit*) the finest divisions of vena cava. The lower ramifications [of vena porta] saturate (*imbibunt*) the orifices (*orificia*) of the vein and impregnate (*potant*) the small branches of the upper vein [the vena cava]. In this way everything touches everything and more efficient digestion takes place by contact. The moistening power of the liver converts the chyle into blood by means of its instrument, heat (Peter of Abano, 1526).

Thus, unlike Turisanus, Peter of Abano supports the view of an open intrahepatic *chyle*-blood movement. Nourishment seeps out of the terminal openings of vena porta into the liver parenchyma and is converted into blood. This in turn infiltrates the fine roots of vena cava and is transported throughout the body. Between the finest ends of vena porta and the thinnest roots of vena cava there appear to be small interstices. Galen's doctrine of the hepatic *sunanastomōseis* is well present in this mediaeval account. Professor in Paris and Padua, the Aponensis knew Greek and Arabic very well. He spent many years in Constantinople and Near-Eastern countries, and had direct access to ancient Greek sources and Arabic translations. He displays a highly developed, exquisite philological skill. Thus, Peter of Abano interprets the spatial arrangement of Galen's *sunanastomōseis* as that of discontinuous structures.

Mondino de' Liucci (c.1270-1326), another student of Taddeo Alderotti, on the other hand, in his *Anothomia* of 1316, affirms Turisanus' concept of a continuity in the structural architecture of the hepatic veins. He neglects the liver *sunanastomōseis* and states that the lobes are formed by separate and scattered veins distributed in the liver substance "like a net" (*ad modum retis*); the space intermingled within the vascular texture is filled with the substance of the liver (Mondino de' Liucci, 1494). The double sets of hepatic veins thus appear to form continuous vascular channels producing a reticular structure. This position is also held by Henry de Mondeville (c.1260-1320), surgical anatomist at Montpellier, who makes no allusion to hepatic *sunanastomōseis* and describes the course of liver veins in essentially the same terms as Turisanus uses (Henry de Mondeville, 1892). Both Mondino and Henry de Mondeville advocated a pragmatic approach to medical issues and actively participated in the practise of human dissection, which began to develop in the academic medical *studia* in the first decades of the fourteenth century (Siraisi, 1990; Carlino, 1994).

In the second half of the fourteenth century, in the late Middle-Ages, the Italian philosopher and physician Jacobus Foroliviensis (Jacobus della Torre) (c.1364-1414) portrays an open organisation of intrahepatic blood flow. He is the representative of a more theoretical and philosophical approach to scientific problems. In the *Expositio et quaestiones in primum canonem Avicennae*, he writes that food enters the invisible channels (*per poros insensibiles*) of *vena chylis* after being excreted from the invisible *capillares* of vena porta, like seeping sweat (*resudando*)(Jacobus Foroliviensis, 1547). Professor of logic and medicine at the universities of Padua, Bologna, Ferrara and Siena, Jacobus was well acquainted with Arabic medical literature and Ibn Sinā's reception of Galen's work.

THE RENAISSANCE

In 1502, Gabriele Zerbi (c.1445-1505), professor of medicine and logic at the universities of Bologna and Padua, writes that "the branches of vena porta ascend toward the convex part of the liver and run to meet (*occurrunt*) the subdivisions of vena cava, which arises in the convex part of the liver. Its branches descend to encounter (*descendunt et obviant*) the branches of vena porta" (Zerbi, 1502). Zerbi's position is ambiguous. He does not openly admit that there is a structural connection between the two sets of veins in the liver parenchyma. He does, however, assert that these vessels "meet" or "encounter" each other. In this way, he seems to acknowledge Galen's intrahepatic *sunanastomōseis* as a kind of continuous structure. In 1523, the Bolognese professor of anatomy and surgery Jacopo Berengario da Carpi (c.1470-1530), in his *Isagogae breves*, seems to explicitly advocate the concept of a close, structural connection between the hepatic branches of vena cava and vena porta. He states that "the thinnest branches of vena cava are joined or united (*coastomantur seu uniuntur*) with the branches of vena porta" (Berengario da Carpi, 1523). Here there is no simple opening of minute orifices between the smallest extremities of the hepatic veins but a true anatomical union. This view is accepted by the Venetian physician and anatomist Nicolaus Massa (1485-1569), who in 1536 claimed that "the thinnest branches [of *vena chylis* or vena cava] are actually united (*uniuntur de facto*) with the branches of vena porta" (Massa, 1536). Massa's assertion is strengthened by the addition of the locution '*de facto*' to '*uniuntur*'. He also provides experimental support for his statement. For, after soaking and boiling liver preparations – he explains – the tissue substance is easily detached from the veins; in this way the complex venous network of the liver parenchyma becomes clearly visible. With Massa we again encounter the concept of the hepatic venous network conceived by Mondino, which implies the existence of a continuous structural connection between the terminal branches of vena porta and vena cava.

We now come to a fundamental step in the development of the concept of anastomoses as closed, continuous structures. Following the model conceived by Berengario and Massa, Andreas Vesalius (1514-1564), in the 1543 edition of *De humani corporis fabrica*, adopts the idea that those subdivisions of vena cava which extend into the liver turn towards the branches of vena porta. In this way, the minute branches of both veins "close tightly together by small orifices" (*osculis inter se connivent*)(Vesalius, 1543). Accordingly, the extremely small vessels which form the roots of vena cava, receive (*transsumant*) the blood produced by the liver in the branches of vena porta and transport nourishment throughout the body. In the second edition of the *Fabrica* of 1555, Vesalius adds this phrase: "and in many places they [the extreme branches of vena porta and vena cava] meet (*congredi*) and unite as continuous structures (*continuari*)" (Vesalius, 1555). Vesalius' passage is crucial because he postulates, with all the force of his authority, the existence of tightly coupled orifices (*oscula*) that structurally connect the terminal subdivisions of vena porta with the terminal segments of vena cava. These *oscula* allow the establishment of a continuous, uninterrupted vascular organization. The word '*oscula*' supersedes the terms *anastomōseis* or *sunanastomōseis* and will become increasingly important in the future history of these entities. The old Greek terms seem now apt to acquire the meaning of a structural union.

Despite Vesalius' theorem, the questions of the mutual relationship between the venous segments in the liver parenchyma and the structural nature of hepatic anastomoses were still the subject of debate among anatomists around the middle of the sixteenth century. Humanistic medicine experienced an increasing revival in the first half of the sixteenth century thanks to the work of scholars, translators and book publishers, who made Galen's writings more widely available, and in a more complete and accurate form than before (Bylebyl, 1979). In addition, the recovery of the original Greek text of Galen's treatise *De anatomicis administrationibus* gave anatomy a whole new significance in academic medicine. Matthaeus Curtius' approach to the problem is much more cautious. Curtius (c.1475-1542) was a Galenist of strict observance and the prototype of the humanistic physician. In his *In Mundini Anatomen Explicatio* of 1550, he raises the question whether "the veins supplied by the vena porta are connected (*iungantur*) with those which proceed from vena cava" (Curtius, 1550). There is uncertainty (*dubitatio*) – he says – whether the *capillares venae* derived from the two principal veins would join together (*iungantur inter se*) in the liver flesh, so that what is contained in the former is conveyed into the latter. It is indeed clearly evident that the nourishment attracted by the portal vein flows in the *capillares venae* and passes through them into the vena cava. This may be done – Curtius explains – in two ways. Either there is a direct connection between the ends of the capillary veins (*venae capillares simul iungantur*), or, if the veins are not connected (*venae quidem non iungantur*), the nourishment is poured indirectly through the spongy flesh in the liver (*per porositates iecoris*) into the capillaries of vena cava. Curtius then quotes Galen's passage from *De locis affectis*, and concludes that "it is not possible to deduce from Galen's words whether [the veins in the liver] are reciprocally connected or not". This conclusion is taken up by the pupil of Realdo Colombo, the Spanish anatomist Juan Valverde de Hamusco (born c.1525), who in 1559 states that the blood passes from vena porta into vena cava "either by union (*congiungendosi*) of the heads of one root with the heads of the other root, or by percolation (*risudando*) of the blood into the substance of the liver and then into the roots of the great vein" (Valverde de Hamusco, 1559).

In 1571 Andrea Cesalpino (1524/25-1603) published his *Quaestiones Peripateticae*. According to the Aretine philosopher and physician, the blood moves from the arteries to the veins through abundant reciprocal '*oscula*' which "the ancient Greeks called anastomoses" (*Graeci*

Anastomosim vocant). These *oscula* are distributed all over the body, not only in the pulmonary vessels but also in the systemic blood vessels. "Everywhere – he writes – veins are connected with arteries" (Cesalpino, 1571). These small orifices (*oscula*) – he adds – are present all along the course of the veins and arteries (Cesalpino, 1571). He does not dwell on the exact architecture of such *oscula*, but it is clear from his description that he regards them as true anatomical constructions, involving a structural continuity of parts. This may be regarded as a consequence of Cesalpino's natural philosophy. He was, in fact, an intransigent Aristotelian, and maintained, in accordance with the Stagirite doctrine, that blood was to be found only within the heart and vessels. Outside the vessels – he argued – no blood was conceivable. This implies a close union of the *oscula* to prevent the escape of blood from the vessels. This point is clearly recognised in the section dealing with the hepatic vascular system. Here Cesalpino states that there are invisible "connections" (*nexus*) between vena porta and vena cava (Cesalpino, 1571). The overall structure, however, is that of a continuous venous network. Galen was mistaken – proclaims Cesalpino – when he considered the relationship between the last branches of vena porta and vena cava to be discontinuous. Cesalpino provides an attractive explanation that accounts for Galen's error. In Galen's experiments, animals were not killed by strangulation, as previously recommended by Aristotle, so that the connection of the hepatic veins was consequently lost. Thus, Galen's *sunanastomōseis* were discontinuous, interrupted structures. On the other hand, in Cesalpino's opinion blood always flows within the vessels. If it drips out of the vessels, it is inevitably subject to putrefaction or agglutination (Cesalpino, 1571). It is impossible, therefore, for the blood of the liver to escape from the veins and re-enter them. The hepatic veins, therefore, consist of a continuous vascular system. This can be clearly seen in accurately washed liver specimens when the blood has been completely removed: an extremely fine texture of the vascular networks (*veluti retis*) is observable.

In 1593 Cesalpino published the *Quaestiones Medicae*, together with the second edition of his *Quaestiones Peripateticae*. In this work, Cesalpino formulates interesting claims. He states that during sleep and in the course of arm ligature, much blood flows from the arteries to the veins through the *oscula*. In particular, he says that in this last condition, "the veins are so much connected (*adeo copulari*) with arteries through *oscula*, that as soon as the vein is cut, the darker venous blood gushes out first, than the more brilliant arterial blood" (Cesalpino, 1593). Here Cesalpino vividly describes a so-called thought experiment (*per imaginationem - Gedankenexperiment*). Venous blood never becomes arterial and always keeps its dark colour. However, Cesalpino's assertion underscores the role he attributes to the *oscula* that connect the arteries to the veins. In summary, Cesalpino conceptualises anastomoses as real, continuous anatomical structures through which blood flows from arteries to veins.

A further chief contribution to the shaping of a new concept of anastomoses was made by the anatomist Arcangelo Piccolomini (1525-1586). Piccolomini died in 1586, when his *Anatomicae Praelectiones*, a rich collection of original anatomical observations, were published. In the chapter devoted to the structure of the liver, in describing the distribution and behaviour of the branches of vena porta and vena cava, he writes:

Both in raw and cooked livers of larger animals, in which everything is more clearly seen than in smaller ones, I have looked very carefully for connections and adhesions of their [the veins] extremities, but still I have never been able to detect them. The roots of both veins are indeed scattered over the substance of the liver without any order, and in many places they do not come into mutual contact (*sese non tangunt*). But when the roots touch (*se tangunt*), it is not along a longitudinal axis, but along a transverse plane. In this way they are seen to form right angles. Indeed, so many roots of vena porta do not penetrate (*infigunt*) with their extremities into the terminal points of vena cava, but into the middle parts of its roots. Similarly, the numerous roots of vena cava, with their terminal segments, do not enter (*infigunt*) into the terminal points of vena porta, but into its roots in the middle parts. In this way the blood flows from the roots of vena porta to the roots of vena cava (Piccolomini, 1586).

He also asserts that the blood is produced in the ultimate branching segments of vena porta, because they are exceptionally more numerous and sensitive than those of vena cava. In summary, Piccolomini contends that (i) there are no mutual end-to-end connections between the branches of vena porta and those of vena cava; (ii) the junctional attachment between these vessels does not run along their longitudinal axes; and (iii) the chyle does not emerge from the roots of vena porta in the liver parenchyma to be absorbed by the roots of vena cava once it has been converted into blood. He strongly maintains that the link between the two sets of veins occurs by means of connections which run along transverse axes. Piccolomini calls these connections "anastomoses of the roots" (*harum radicum anastomoses*), or "kisses" (*exosculationes*) between vessels, or "openings from the one to the other" (*ex alterutris in alteras apertiones*)(Piccolomini, 1586). Remarkably, he improves his textual account with an instructive figure sketching the course of vena cava, vena porta, their branches, and root extremities. This drawing depicts some terminal segments of vena cava entering the middle of some of the roots of vena porta, as well as some terminal segments of vena porta entering the middle roots of vena cava. The caption defines these short connections as "insertions and anastomoses" (*insertiones et anastomoses*). Three conclusions can be drawn from Piccolomini's illustration. They are of overriding importance for the conceptual shift of anastomoses from purely functional states to genuine structural entities. Thus, according to Piccolomini, i) the term anastomoses denotes proper anatomical arrangements involving continuity of parts. Moreover, ii) anastomotic vascular connections are not end-to-end but end-to-side connections. Finally, iii) Piccolomini's doctrine implies, in contrast to Galen's assumption, that anastomoses are not merely invisible, imperceptible structures that elude the gaze and can only be theoretically surmised by the eye of reason, but well-documented and observable vascular pathways. It is difficult to say what Piccolomini actually observed in his experimental studies because in the human liver and in the liver of mammals there are usually no end-to-side or other types of visible connections between the branches of the vena porta and the roots of the vena cava. Modern imaging techniques have shown that multiple intrahepatic communications between the branches of the porta and hepatic veins are rare disorders. Interestingly, they occur more frequently in the right lobe of the liver. Most often, they are congenital defects, but acquired intrahepatic portosystemic shunts resulting from trauma or portal hypertension have also been described (Park et al., 1990; Corness et al. 2006). William Harvey, as we will see in the next section, openly admitted that he was unable to recognize anastomoses in the liver, although he spent much time and effort trying to find them. Anyway, Piccolomini's theoretical contribution was fundamental in shaping the modern concept of anastomoses.

THE SEVENTEENTH CENTURY

The seventeenth century begins with a fundamental contribution to the disclosure of the structure of anastomoses by the Swiss anatomist, physician and botanist Caspar Bauhin (1560-1624), professor of practical medicine at Basel. In 1605 he published a first-rate book, *Theatrum Anatomicum*, in which he supports Piccolomini's conception of hepatic anastomoses as a continuous connection between the roots of vena porta and those of vena cava (Bauhin, 1605). Specifically, the roots of both veins join along a transverse plane of incidence (*per transversum per Anastomoses commissis*), according to an end-to-side connection pattern. Bauhin claims that in the concave part of the liver the branches of vena porta are abundant and outnumber those of vena cava. The reverse is the case in the convex part of the organ. Thus, in the lower part of the liver there are many more branches of vena porta which penetrate (*infigunt*) the middle segments of the roots of vena cava and in the upper part the reverse is true. The formation of blood moves from the concave part of the liver to the curved surface of the organ through a "*mirabilis plexus*" formed by the origins of vena porta and those of vena cava. The blood flows through these anastomoses, and then passes throughout the body. The description of a "*mirabilis plexus*" in the liver parenchyma – that is the presence of an interlaced vascular network formed by the origins of the vena porta and those of vena cava – is an interesting conceptual novelty in the development of the idea of modern anastomoses and represent a further step in the approach to the truth. Without any microscopic support, one could only imagine the structural configuration of invisible blood vessels. This implies a method of reductionism leading to an abstract simplification of the real vascular texture of the part. Bauhin presents the same illustration that was published by Piccolomini. It is particularly interesting that he mentions a unique giant anastomosis (*peculiaris et insignis Anastomosis*), which imitates a canal (*quae canalem aemulatur*) and presents itself as a common, continuous duct (*veluti communis et continuus ductus*) allowing the penetration of a rather large stylet. It is possible that this is a *ductus venosus Arantii* which has not yet been closed. Bauhin provides another illustration in which this conduit is clearly seen (Bauhin, 1605). Furthermore, Bauhin extends the scope of the term 'anastomoses' to include the vascular connexions (*extremis iunguntur*) on the anterior wall of the trunk between the branches of the internal mammalian veins and those of the inferior epigastric veins (Bauhin, 1605). These anastomoses are located around the umbilicus (*circa umbilicum per Anastomoses*) and provide connexions between the matrix and the *mammae*, a subject of great importance in the Hippocratic and Galenic mechanism of lactation. In modern texts of anatomy, these connexions are still considered part of the anastomotic apparatus between superior and inferior vena cava (Standring, 2005). In summary, Bauhin's contribution to the history of the semantic shift of the term *anastomōsis* from a purely functional meaning to a fixed, structural significance is highly relevant. In the Swiss anatomist's text, anastomosis denotes an elongated, continuous *trait d'union* representing a common structure between two main vessels. Bauhin's opinion is shared by his pupil, the Danish anatomist Caspar Bartholin (1585-1629). In his *Anatomicae Institutiones corporis humani*, first published in 1611, he rejects the concept that an anastomosis is a relationship between separate vessels and defines intrahepatic 'anastomoses' as "conjunctions (*conjunctiones*) between the roots of vena porta and cava" (Bartholin, 1626). They can follow either an end-to-side or an end-to-end union pattern.

Fourteen years later, in 1625, the German anatomist Caspar Hofmann (1572-1648), professor of medicine at Altdorf, published a Commentary on Galen's *De usu partium*. Hofmann was an Aristotelian, strongly influenced by Cesalpino. One of his main beliefs was the nourishing property of arterial blood. Not only venous blood but also blood flowing in arteries imparts nutritive power. In this perspective, *anastomōseis* play a crucial role in Hofmann's physiology. Venous and arterial blood "communicate through reciprocal openings of the vessels" to produce one type of blood (Hofmann, 1625). He even asserts that "it is the purpose of nature to connect (*conjungere*) veins with arteries" (Hofmann, 1625).

[Nature] – he explains – connected (*coniunxit*) veins in which thicker blood flows with arteries in which finer blood runs through *anastomōseis*. In this way a unique quality [of blood] is produced in each part to be nourished, as similar as possible to the quality of the parts themselves, by mixing both kinds of blood (Hofmann, 1625).

This fundamental role of anastomoses in bodily processes is achieved by anatomical communications that structurally connect veins with arteries (*conjunctiones venarum et arteriarum*)(Hofmann, 1625). With regard to hepatic anastomoses, Hofmann states that the orifices of the terminal branches of vena porta and vena cava are reciprocally connected (*sibi invicem iungi*)(Hofmann, 1625). In this way – Hofmann asserts – a style which has penetrated the vena porta enters the region of the vena cava. Leaving aside the doubtful experimental authenticity of this last proposition, we can safely conclude that anastomoses in Hofmann's doctrine are genuine constructions characterised by physical continuity between connected parts.

Changing conceptions on the structure of anastomoses have critical importance for the development of the theory of circulation. In the XI chapter of his *Exercitatio anatomica de motu cordis et sanguinis in animalibus*, William Harvey (1578-1657) postulates a circular flow of blood, moving ceaselessly from the arteries to the veins either directly through anastomoses (*per Anastomosin immediate*), or indirectly through tissue permeability (*mediate per carnis porositates*), or in both ways (Harvey, 1628). In his masterpiece of 1628 he does not bother with structural details about anastomoses. But in the epistle to Paul Marquard Schlegel, dated 26 March 1651, in London, he has occasion to discuss with some minutiae the physical architecture of anastomoses, a subject he had scarcely touched upon before in *Exercitatio anatomica prima*, addressed to Jean Riolan (1580-1657) in 1649 (Harvey, 1649; Harvey, 1958). Riolan indeed claimed that blood in the great vessels flows through visible anastomoses (*per multas anastomoses vasorum*) from the arteries into veins (Riolan, 1649a; Riolan, 1649b). By the time of *Exercitatio anatomica prima* and the letter to Schlegel, Harvey had already abandoned the idea that anastomoses would play a significant role in the transfer of blood from the arterial to the venous compartments. He admits that he had failed to identify these elusive structures. Despite intensive efforts, he confesses to having found something comparable to (*quod aequipollet*) anastomoses in only three vascular compartments, namely, the cerebral plexus vessels, the gonadal vessels, and the umbilical vasculature. He admits that the anastomoses discovered at these sites have a different structure from that imagined by the Ancients. Indeed – he says – anastomoses had always been thought of as bidirectional transit devices. But the theory of blood circulation implies a one-way passage of the blood. From this point of view, he reports an important vivisection experiment. When in a living animal the aorta is ligated at its origin and the blood is withdrawn from the arteries, the veins nevertheless remain filled up with blood, because it cannot flow back into the arteries either spontaneously or forcibly. Harvey hypothesised that the arterioles enter the wall of the venules at an angle and run a short distance between their coats, much like the entrance of ureters into the wall of the vescica or of the bile duct into the wall of the duodenum. The reflux of blood from the venules into the arterioles is thus prevented. He also furnishes experimental proof of his statement. In the *Exercitationes de generatione animalium* Harvey reports that insufflation of air in the ovarian arteries causes dilatation of the ovarian veins, which is due to a transfer of air from the arteries to the veins, possibly by anastomoses (Harvey, 1651). The reverse, however, never occurs. He thus admits that under certain circumstances (*interdum*) there is a direct (*immediate*) transfer of blood from arteries to veins through anastomoses. The term anastomoses should be avoided, however, because it implies that "vessels may open equally on both sides, so that blood may pass freely on this side and on that" (Harvey, 1958). Harvey concludes his thesis in the hope that the old term 'anastomoses' will be finally discarded. It is an unfamiliar, inappropriate word for the purpose of circulation.

CONCLUSION

This study addresses a neglected piece of scientific and medical history, namely the controversy about the actual structure of blood vessel communications and the changing meaning of the term '*anastomōsis*' from Antiquity to the Seventeenth century. It is concerned with the distinction between the early conception an of open, functional state, as formulated by Erasistratus of Ceos, Aretaeus the Cappadocian, Galen and Oribasius, and the later developed notion of a closed, anatomical structure, as it prevailed and established in the Middle Ages and the Renaissance, into the modern period. The different views of ancient physicians on the structure of the *anastomōseis* in Antiquity cannot be properly reconstructed because of the great lack of preserved medical records. However, the testimony of the pseudo-Aristotelian author of *De spiritu* and the cautious approach to the problem of the structure of intrahepatic *anastomōseis* advocated by Galen himself in *De facultatibus naturalibus* may reflect an ongoing, unresolved controversy over the actual configuration of blood vessel connections. Nonetheless, the open and purely functional conception of the *anastomōseis*, as developed by Erasistratus, appears to have ultimately prevailed in Antiquity according to the extant record. Galen himself apparently adopted it. The first semantic shift of the term '*anastomōsis*' seems to have occurred in the Islamic cultural sphere toward the end of the ninth century and the beginning of the tenth, thanks to the work of al-Rāzī. He postulated an uninterrupted architecture of venous vessels in the liver parenchyma, claiming that the extremities of the porta and cava veins "are connected". In the Western world, Petrus Turisanus, Mondino de' Liucci and Henry de Mondeville, were the first to conceive of hepatic vasculature as a continuous structural network in the early fourteenth century. In the late Middle Ages and Renaissance, there was a lively debate on the structure of *anastomōseis* between representatives of the Arab-scholastic tradition and the humanistic tradition in the context of the evolving practice of human dissection. This debate involved such figures as Peter of Abano, Jacopo Berengario da Carpi, Nicolaus Massa, Matthaeus Curtius and Juan Valverde de Hamusco. Major contributions to the shaping of a tight, structural concept of *anastomōseis*/anastomoses were made by Vesalius, Cesalpino, Piccolomini and Bauhin. Harvey put an end to it. He linked the question of the spatial configuration of these entities with the question of blood circulation. The letter to Schlegel is an important document that attests Harvey's historical and linguistic awareness of the origin and concept exhibited by the term '*anastomōsis*'. For the Ancients, *anastomōseis* were conditions in which vessels faced each other mouth to mouth. Harvey states that this circumstance does not exist. Anastomoses are physically interconnected structures, with the terminal segments of arterioles penetrating the wall of the venules and running transversely for a short tract in order to prevent the reflux of blood. The conceptual and semantic shift from an open vascular state to a tight, structured anatomical connexion is complete. From now on, the word anastomoses will symbolise direct connections between blood vessels.

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IJAE
ITALIAN JOURNAL OF
ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Orsini, D., Martini, M., Ottoboni, S., Aglianò, M., Franci, D., Lorenzoni, P., & Saverino, D. (2024). Drawings, artifacts and anatomical preparations: the collections of the Senese Museums for teaching Anatomy yesterday as today. *Italian Journal of Anatomy and Embryology* 128(2): 75-83. https://doi.org/10.36253/ijae-15378

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Drawings, artifacts and anatomical preparations: the collections of the Senese Museums for teaching Anatomy yesterday as today

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Abstract. The scientific museology highlights the importance of the specialized museum collections of anatomy as tools for teaching and scientific communication. The cultural heritage, deriving from research and study activities, have been preserved over the centuries They are not simple goods to be exhibited, but are dissemination means of scientific culture and specialized teaching at university level. In fact, anatomical tables, models and preparations of the Senese museums are still used for teaching anatomy, as well as for dissemination activities to a large non-specialized audiences. The anatomical collections and the musealization of new findings are proposed as active means of communication and teaching, of health education and research on the biological history of man. Through their collections, the universities museums of anatomy allow to carry out empirical studies of a historical nature. In addition, the evolution of technologies can help the knowledge of the human body. The historical re-reading of anatomy by means of the goods present in the museums of human anatomy, the study of the technical and practical knowledge at the base of such finds, the possibility of using them for educational purposes alongside the most up-to-date tools that technology allows us to have available, are producing a new interest in the discipline that, overcoming the limits of a specialized knowledge, can acquire the value of a form of historical communication of the human body that can also be communicated to an inexperienced audience, as well as to students of medical degree courses and health professions.

Keywords: anatomy, museums collections, heritage, teaching.

INTRODUCTION

Anatomical museums, often found in many ancient universities, can be considered as true three-dimensional works of anatomy, where the human body is displayed in its normal state or in the pathological aspects that can characterize it. In anatomical museums, through human remains, drawings, and models, the structures and morphologies of the human body can be studied as an expression of the concept of normality in relation to possible pathological evolutions and the "monstrosities" of the teratological collections that are often found in 19th-century museums.

In fact, we should not forget that some collections of anatomical preparations have had not only an important role in teaching but also a specific social function: through what Italo Calvino has defined as the "pedagogy of the macabre" (1), showing specific pathological conditions meant to warn the population of the consequences of reprehensible behaviors, which society had to eradicate in favor of a wise and morally accepted conduct. For this reason, as an educational warning, preparations and models were exhibited that represented the effects on the body caused by syphilis, alcoholism, and tuberculosis.

However, the assets preserved in anatomical museums are above all the result of research on the biological history of man, the educational tools, and means for historical-medical dissemination.

The recent scientific museology particularly highlights the importance of specialized anatomical museum collections for teaching and scientific communication, today as then.

In this sense, the cultural assets that derive from the research and study actions of universities, which have been preserved for centuries, cannot be longer considered as simple assets to be conserved, studied, and possibly exhibited, but they assume the value of means of dissemination of scientific culture and specialized teaching at university level, as well as instruments for outreach activities to a wide range of non-specialist audiences.

THE ROLE OF ANATOMICAL MUSEUMS IN HISTORY

Although the study of human anatomy began officially in the 14th century and then more widely in the Renaissance, some physicians had already begun to make anatomical preparations for teaching purposes, preserving them in private scientific cabinets. It was with the Enlightenment, however, that these collections took on value for civil society, thus giving rise to the idea of a museum as a public good.

The foundation of pathological anatomy was fundamental to the anatomical museums birth and evolution. This is the time and the reason why anatomy becomes essential for clinical practice, determining a new and stronger interest in the study of the human body.

Actually, the history of the objects found in these museums has more ancient roots. Indeed, they can be traced back to the Renaissance period, when "the disciplines of the natural sciences based on description and observation (zoology, botany, paleontology, physics for many aspects and, above all, anatomy) had a development directly correlated with the development of representation techniques" (2). And immediately afterwards, in an attempt to give a new impetus to university studies, they became part of a teaching method based on observation and practical demonstration.

Drawings, preparations, and models soon proved to be valid teaching tools, useful for showing and memorizing the details of the body's structures. And if the preparations show the high professionalism of the technicians employed in the anatomical cabinets and institutes, the drawings and models, that represent the morphology of the bodies, are in substance artistic artifacts (3). After all, "science has always made use of the creativity and the art, which have allowed it to disseminate in a simple, spectacular, and fascinating way a knowledge that is often difficult to construct. In a strategic combination, art and science have aligned themselves over time to produce teaching aids that are in reality (also) very refined artworks, as faithful as possible to the scientific reality" (4). In this way, in the absence of organic material preserved as preparations, students could avail themselves of effective and faithful representations in wax, terracotta, or ceramic models, which replaced cadavers as teaching tools for learning anatomy or were shown as semiophors of the history of diseases in the anatomical-pathological collections.

To testify to the importance of the teaching function of the objects preserved in anatomical museums, we report the statement made by the anatomy professors of the University of Siena in 1882 on the occasion of the award at the X Congress of the Italian Medical Association for having exhibited "several macroscopic preparations referring more especially to the nervous system, and with various other microscopic preparations of embryology". These preparations fully correspond to the purposes of the School of Anatomy in relation to "the needs of teaching and science" (5). In the collection of the Sienese Anatomical Museum - as can be read in the *Relazione sull'Università di Siena e sugli stabilimenti scientifici* for the academic year 1886-1887 - "every system of the human organism is represented and widely and also according to age. About a thousand skulls, very many brains; admirable preparations of entire regions with their respective nerve systems and the relationships between the different nerves that take part in them. There are than a microscopic and an embryological collection" (6).

While the teaching and dissemination function of such collections is absolutely accepted for the past, according to several scholars the modern teaching systems, which make extensive use of technology, make the use and in some ways the preservation of anatomical specimens no longer essential for these purposes.

The experiences carried out in many anatomical museums, such as the Gordon Museum of Pathology in London (7), which is certainly a *best practice*, but also some university museums such as the Leonetto Comparini Anatomical Museum which is part of the Sienese University Museum System, disprove this vision, reaffirming the strong and fundamental educational value of such cultural assets (8-10).

CULTURAL ASSETS AS TEACHING TOOLS IN THE SIENESE ANATOMICAL MUSEUMS

Anatomical museums are repositories of invaluable specimens that shed light on the intricacies of the human body. This has been their primary function for centuries, and it remains a cornerstone of education at the University of Siena.

Anatomical drawings and preparations derived to the practice of dissection for educational purposes. This approach, pioneered by Mondino de' Liuzzi (1275-1326) (11) in the early 14th century, revolutionized the transmission of knowledge within universities. The renewed emphasis on dissection led to the creation of teaching aids that could be utilized during periods when dissection was not possible. Anatomical tables, in particular, were deemed more effective than written texts, allowing individuals across different locations to observe what could otherwise only be seen through dissection. Similarly, preparations and models served as substitutes for direct observation of the cadaver (12).

This article highlights the crucial role of anatomy museums in preserving and disseminating knowledge about the human body. It emphasizes the value of anatomical drawings, preparations, and models as educational tools, both in the past and in the present. These specimens serve as invaluable resources for understanding the human form and its complexities, bridging the gap between theoretical knowledge and direct observation.

The models

Among the many fascinating anatomical specimens housed in the University of Siena's Anatomy Museum, one particularly stands out: a papier-mâché ear model, larger than life-size, dating back to 1877. This remarkable creation is attributed to the French anatomist and naturalist Louis Thomas Jérôme Auzoux (1797-1880), as indicated by the signature on the model itself: "*Auzoux Doct.re fecit anno 1877.*"

The model's brilliance lies in its intricate design, comprising nine meticulously crafted, detachable parts. This feature makes it an exceptional educational tool, allowing students to gain a comprehensive understanding of the human ear's anatomy. By carefully examining each component, they can visualize the intricate structures of the outer, middle, and inner ear, gaining a deeper appreciation for the complexities of this vital sensory organ.

In his search for more durable, affordable, and user-friendly anatomical models alternative to those made in wax, Louis Thomas Jérôme Auzoux (1797-1880) embarked on a revolutionary endeavor in the 1820s. He developed a novel technique, crafting models that were not only less fragile than their wax counterparts but also easily disassembled to reveal the intricate details of the human body.

These innovative models, properly named "anatomies clastiques" (clastic anatomies), drew inspiration from the Greek word "*klastos*" meaning "broken in pieces." This model has in itself the very characteristic of the definition: the ability to be dismantled, allowing

Figure 1. Thomas Jérôme Auzoux, *Ear Model*, 1877 (Leonetto Comparini Anatomical Museum - University Museum System of Siena).

students to acquire a complete understanding of the anatomical structures represented, from the epidermis to the bones, from the blood vessels to the nerves (13).

Auzoux's ingenious method involved a unique blend of paper pulp, glue, and cork dust, carefully pressed into paper-lined molds. For simpler components, he employed plaster molds coated with layers of colored paper, meticulously soaked in glue. For articulated models, Auzoux devised a specialized paste that, upon drying, transformed into a robust material capable of supporting fasteners, hinges, or even metal frames for larger models.

The ear model, crafted with meticulous attention to detail, showcases the versatility of Auzoux's modeling techniques. Its larger components are meticulously constructed using the traditional papier-mâché method, involving layers of paper pulp carefully pressed into molds (13). This technique lends the model its durability and lifelike appearance.

For the finer details, Auzoux employed a range of materials, each selected for its unique properties and ability to accurately represent the intricate structures of the ear. The facial cranial nerve, a critical component of the auditory system, is crafted from a combination of wood and plaster, ensuring its sturdiness and intricate details. The tympanic membrane, the delicate barrier separating the outer and middle ear, is meticulously recreated using a double layer of gelatinous material. Its translucent quality mimics the membrane's delicate nature, allowing students to visualize its role in sound transmission. The larger arteries, essential for transporting blood to and from the ear, are constructed using a unique technique. Straw cords are carefully wound around a central metal core, providing a sturdy foundation. This structure is then coated with a layer of paper, creating a realistic representation of the arteries' appearance and texture. Each element is carefully chosen and meticulously crafted to ensure an accurate and visually appealing representation of the ear's anatomy.

This innovative approach revolutionized anatomical modeling, offering a multitude of advantages over traditional wax models. The "anatomies clastiques" were not only more durable and economical but also far more versatile, allowing for a deeper exploration of the human body's sophisticated structures.

In 2018, Auzoux's exquisite ear model embarked on a meticulous restoration, guided by the skilled hands of experienced conservators. The model, having endured years of careful study and admiration, bore the marks of time: a layer of grime obscuring its original vibrant hues. With utmost care, the conservators undertook their task, meticulously cleaning each component, gently removing the accumulated dust that had dimmed the model's splendor. Their attention to detail ensured that the original painted surfaces remained intact, preserving the integrity of Auzoux's art. Once the cleansing process was complete, the conservators turned their focus to reinforcing the areas that had suffered from the passage of time. They carefully consolidated areas where the paint film had lifted, stabilizing these delicate structures and preventing further damage. Additionally, they addressed areas exhibiting surface craquelure, fine cracks that threatened to ruin the model's beauty. With the structural integrity restored, the conservators embarked on the final phase of the restoration: the retouching process. They employed the delicate technique of watercolor pointillism, meticulously applying tiny dots of color to match the original colors. This approach allowed them to subtly blend the retouched areas with the remaining original paint, ensuring that the model's authenticity remained intact.

The meticulous restoration of Auzoux's ear model extended beyond aesthetic considerations, addressing the fundamental need to preserve a cultural treasure while ensuring its continued use for educational purposes. With extreme care, the model has been reintroduced into the ambit of teaching, serving as an invaluable tool in the Otorhinolaryngology specialization school, in addition to captivating audiences at various public events.

This remarkable restoration exemplifies the delicate balance between safeguarding cultural heritage and ensuring its relevance in the present day. The ear model, once again a testament to Auzoux's artistry and the conservators' expertise, stands ready to inspire and educate future generations of medical professionals and the general public alike.

Also produced by Azoux, deriving from the collections of what was once called Physiological Anatomy, a model of eye in painted papier-mâché, jute, metal and glass, is preserved in the Museum of Medical Instrumentation, other reality of the Museum System of the University of Siena (Figure 2).

In the same Museum, nine educational obstetric pottery testify to the important attempt made in Siena, in the second half of the 18th century, to offer basic education to women who were or would have been midwives. These pieces, commissioned by Jacopo Bartolommei (1708-1782), the master of parts and lecturer of Obstetrics at the University of Siena, based on the model of those made in Bologna by Giovanni Antonio Galli (1708-1782). Originally the models were 40, of which 8 were life-size, 19 "half the natural size", four of about a quarter (8). They illustrate the various presentations and fetal situations, both from the physiological and pathological point of view (14). Skilfully restored, they are still

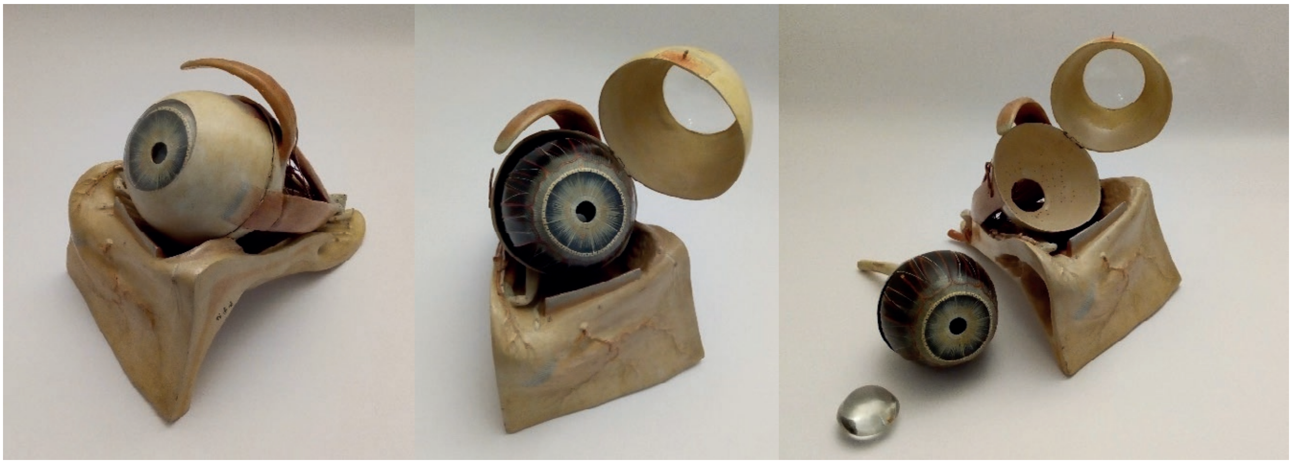

Figure 2 a, b, c. Thomas Jérôme Auzoux, *Model of eye*, second half of 19th century (Museum of Medical Instrumentation, at the Museum System of the University of Siena).

Figure 3. Educational obstetric pottery, second half of the 18th century (Museum of Medical Instrumentation, at the Museum System of the University of Siena).

used today to explain the history of birth and the evolution of obstetric practice in internships dedicated to medical students (Figure 3).

The anatomical drawings

The contribution of anatomical tables to the teaching of anatomy is still considered fundamental. Every student joins the study on the book to the vision of the tables on the anatomical atlas. It was in Siena that the revolutionary idea of an anatomical atlas with life-size figures was born. The scholar who had this idea and realized it was Paolo Mascagni (1755-1815) (15).

In his work entitled *Anatomia universa* (16-19) extraordinary in conception and commitment, Mascagni create detailed anatomical tables reproducing the human body in real size, and in extremely detailed way. A universe of vessels, muscles, bones, organs, whole and dissected dedicated to medical students. The attention to the real proportions testifies how much Mascagni distanced himself from the scholars who had preceded him, while knowing and respecting their work (16-19).

The *Anatomia Universa* by Paolo Mascagni is still today, 200 years after its publication, a perennial proof of his ability as a scholar and teacher and his desire to contribute to the formation of a new medical class.

Next to the tables of Anatomia Universa, now exhibited in the Natural History Museum of the Accademia dei Fisiocritici of Siena, the University preserves and makes available to students and visitors of the Anatomical Museum the superb tables of the *Vasorum lymphaticorum historia et ichnographia* (20), published by Mascagni in 1787. For the first time in the history of Medicine lymphatic system are described and presented in special drawings.

In addition of Mascagni's works, teatrise by another great scholar of anatomy, Antonio Scarpa (1752-1832), and about 600 more modern anatomical tables, some of the late nineteenth and other twentieth century are conserved in the Museum of Siena. Made on commission of teachers by designers and technicians who over the centuries have assisted the anatomy professor during dissections and lectures, represent a valuable collection of drawings, watercolors and lithographs that faithfully reproduce the apparatuses of the human body and that are still fundamental for teaching.

The anatomical preparations

The last category of didactic tools for the study of anatomy, which more than others suffers from the close relationship with dissection, is that of anatomical preparations. Absolutely preferred by students and those who want study of the human body, they offer a three-dimensional vision of the organ or section of the body that they represent.

In this case, the skill and professionalism of the preparators were extraordinary in creating specimens that can withstand the test of time, using various methods such as drying or formalin preservation.

The intricate craftsmanship and enduring value of anatomical specimens make them irreplaceable treasures in the realm of medical history and education. However, their preservation and restoration pose a complex challenge for museums, demanding a delicate balance between safeguarding these artifacts and ensuring their accessibility to the public. Open dialogue and informed decision-making are crucial to develop strategies that respect the historical significance of these specimens while upholding ethical considerations.

The exhibition of *human remains* demands a heightened level of sensitivity and ethical consideration. The emotional impact on visitors must be carefully weighed and addressed in the design of any display, whether temporary or permanent. Clear communication of the exhibition's purpose is paramount to ensure a respectful and meaningful experience for all.

The work of Gunther von Hagens (21, 22), the inventor of plastination (23, 24), has sparked intense debate in recent years. His exhibitions, showcasing meticulously preserved human bodies, have been lauded by some as innovative approaches to evidence-based teaching while simultaneously criticized by others as commercial spectacles that disregard the sanctity of the bodies displayed.

Also Damien Hirst's artworks, particularly his iconic series of animals suspended in formaldehyde, have garnered both acclaim and controversy. The stark juxtaposition of life and death, the preservation of once-living creatures in a sterile medium, has been interpreted as a profound commentary on mortality, challenging viewers to confront their own fears and perceptions of death (25).

In addition, Bill Viola's video installations (26), often featuring slow-motion sequences of human bodies in water or other immersive environments, have captivated audiences with their ethereal beauty and profound exploration of human existence. Viola's work delves into themes of birth, death, transformation, and the spiritual realm, inviting viewers to contemplate the ephemeral nature of life and the possibility of transcendence.

Anatomical museums, in showcasing their collections of anatomical specimens composed of *human remains*, must be acutely aware that they are handling "culturally sensitive materials" according to ICOM's ethical guidelines (27). This necessitates a thorough and nuanced approach to ethical considerations in the display and interpretation of these specimens.

With the aforementioned ethical considerations firmly in mind, it is important to recognize that anatomical specimens, due to the transformative effects of time and human intervention, no longer fall under the legal definition of cadavers and do not evoke the same sentiments of piety towards the deceased. Nonetheless, these specimens retain their fundamental importance in anatomical education (28).

The Anatomical Museum of the University of Siena preserves and exhibits many preparations. Of particular interest are some preserved in formalin: in ancient glass containers are present fetuses at different stages of maturation, sometimes accompanied by placenta and amniotic sac and various organs including hearts, lungs and encephalia. Interesting a preparation of spinal cord wrapped by the meninges with the emergence of spinal nerves that in the last stretch gather to form the so-called "cauda equina".

Particularly rich is the collection of dried preparations, obtained by injection of sclerosing substances, subsequent staining and sometimes insufflation: these are mainly segments of upper and lower limbs, organs of the digestive, respiratory, cardiocirculatory and urogenital systems (Figure 4).

A peculiarity of the Museum are the craniological collections, prepared directly in the Institute of Normal Human Anatomy. Of the skulls that make up these collections some appear selected on the basis of the theory

Figure 4. Arteries of the stomach, prepartion, second half of the nineteenth century (Leonetto Comparini Anatomical Museum - University Museum System of Siena).

of the "criminal by birth" of Cesare Lombroso (1835-1909), others come from the former psychiatric hospital of Siena. Of great interest is an osteological collection of over 300 skulls of fetuses and newborns dating back to the nineteenth century.

The skulls from the San Niccolò Mental Hospital of Siena are recorded in a Register, kept in the Museum, started in December 1862 by Carlo Livi (1823-1877) who was director of the psychiatric facility from 1858 to 1873 (29). The collection started from a pre-existing nucleus of eight skulls or parts of them, coming from the anatomical collections of the University of Siena.

The craniological collection preserved today at the Anatomical Museum is definitely linked to a research project resulting from an idea presented by Livi and other psychiatrists on September 28, 1862 in Modena at the tenth Congress of Italian Scientists. In this context it was proposed to promulgate a law on mental hospital and to extend the study of psychiatry. Thus, this collection of skulls is part of the Livi's project: to study the bone and endocranial pathologies of the patients of the mental hospital, with the aim of confronting in a new and experimental way with mental pathology. The recording of the skulls was carried out by Livi's students and in particular by Paolo Funaioli (1848-1911) who, starting from 1881, recovered the Register attempting to fully understand the mechanisms of anatomy-pathological and physiological underlying mental pathology. A total of 284 skulls (174 belonging to males, 110 to females) were recorded in the Register.

The collection of the Register is configured for Livi and Funaioli as a tool for studying and enriching psycho-pathological, neurological and anatomo-pathological studies, to meet the needs of new knowledge useful to really affect the care of patients admitted, and therefore on their living conditions.

The Register collection, designed for Livi and Funaioli, serves as a vital tool for research and advancement in the fields of psychopathology, neurology, and anatomo-pathology. The aim is to leverage new knowledge gleaned from these studies to directly improve patient care for those admitted to the facility. Ultimately, this translates to a positive impact on their quality of life.

CONCLUSIONS

The importance of the collections in the museums of anatomy in the teaching of anatomy is evident. Methods and technologies have evolved over the centuries, but the study and understanding of anatomy is always based on direct observation of the human body mediated by tools such as drawings, preparations and models.

Anatomical museums, with their treasure troves of preserved specimens, are not merely repositories of the past; they are dynamic hubs that can rekindle interest in the study of anatomy. By delving into the historical context of these specimens, exploring the technical and practical knowledge behind their preparation, and utilizing them for educational purposes alongside modern technological tools, these museums can transcend the boundaries of specialized knowledge and engage a broader audience.

Anatomical museums are not merely static repositories of the past; they are also dynamic hubs of scientific exploration. The advent of new research methodologies, coupled with the wealth of information contained within these collections, allows researchers to delve deeper into the mysteries of the human body, uncovering hidden secrets and advancing our knowledge of anatomy and physiology.

Despite some criticisms, through innovative exhibitions, interactive displays, and engaging educational programs, these museums are making the wonders of the human body accessible to a wider public, turning on a sense of curiosity and appreciation for the intricate workings of our anatomy.

The result is the need for a reflection from a museological point of view on the exhibition of anatomical collections and on the musealization of new finds: these collections must increasingly be proposed as active tools of communication and teaching, health education and research on the biological history of man, taking into account also the new sensitivity of the public towards human remains that are a fundamental part of the museums of anatomy.

Finally, anatomical museums with their rich collections, educational potential, and capacity for scientific discovery, play a vital role in the future of anatomy. They serve as bridges between past, present, and future, connecting us to our anatomical heritage while illuminating the path forward in our quest to understand the intricacies of the human body.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Fulceri, F., Ryskalin, L., Morucci, G., Soldani, P., Ratti, S., Manzoli, L., & Gesi, M. (2024). Perception of physical therapists towards cadaver dissection: a qualitative survey study. *Italian Journal of Anatomy and Embryology* 128(2): 85-92. https://doi.org/10.36253/ijae-15380

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Perception of physical therapists towards cadaver dissection: a qualitative survey study

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Abstract. A deep understanding of gross anatomy is imperative for healthcare professionals for clinical examination, diagnosis, and safe and effective treatment of their patients. In this regard, satisfactory assessment of musculoskeletal disorders by physical therapists (PT) requires an in-depth knowledge of the morphology, position, and spatial relations of the structures of the locomotor system. Within this frame, traditional anatomical teaching and learning practices alone appear as a limit. Although they represent the mainstay of anatomy education, formal lectures, textbooks, or atlases only provide a theoretical knowledge of gross anatomy. Contrariwise, cadaver dissection offers unique advantages, namely appreciation of 3D concepts of body organization and spatial relationships between anatomical structures, being the most significative ones. Again, hands-on educational experiences can stimulate student interest, increase knowledge retention, and enhance the development of clinical skills. Thus, the use of human cadaver dissection is of paramount importance in the development of PT's core competencies, such as clinical education and reasoning. Therefore, the present study aimed to examine the appraisal and attitude toward the cadaver dissection experience of physical therapists (n=56) attending the postgraduate course in Sports Physiotherapy at the University of Pisa. The one-day human cadaver dissection course was held at the Institute of Human Anatomy of the University of Bologna. At the end of the course, the PT were invited to complete a self-administered questionnaire. All participants agreed that dissection is important, relevant, and highly beneficial to their anatomy education. They also perceived that this educational experience has the potential to improve learning outcomes that are essential to the development of healthcare professionals. The results presented in this study provide preliminary evidence that cadaver dissection is a highly valuable tool for developing fundamental skills and competencies in the training and professional careers of PT.

Keywords: human anatomy, cadaver dissection, hands-on learning, gross anatomy education, clinical knowledge.

INTRODUCTION

Human anatomy is one of the basic medical sciences that has always played a fundamental role in the education and training of medical doctors and healthcare professionals. Indeed, adequate anatomical knowledge is essential for a safe and effective clinical practice, a correct diagnosis, and patient management. Many authors have drawn attention to how anatomical ignorance may be detrimental in surgery, sometimes leading to increased litigation in failure cases (Ellis 2002; Fischer 2002; Goodwin 2002; Singh et al. 2022). Again, poor anatomical knowledge may be a potential negative factor in clinical practice, though a direct correlation with malpractice would be difficult to substantiate (Rompolski et al. 2023). Contrariwise, properly defining the anatomical site of the lesion is crucial for health professionals, including physical therapists (PT), to manage the injury and solve the problem effectively, thus successfully pursuing clinical practice (Estai and Bunt 2016; Ghosh 2017).

When looking at the worldwide educational trends over the last century, it clearly emerges that teaching anatomy has been undergoing reforms for a long time. In particular, with evolving technology, traditional anatomy education, such as didactic lectures and body dissection, has been complemented and, in some cases, superseded by a wide range of novel educational approaches, including plastic models, 3D anatomy atlases, virtual/augmented reality, and many other teaching tools (Peterson and Mlynarczyk 2016; Richardson et al. 2021; Asad et al. 2023; Funjan et al. 2023). As a result, there has been a significant reduction in the amount of time dedicated to traditional cadaveric dissection, which has sometimes also been referred to as an obsolete practice (Memon 2018).

On the other hand, despite the increasing trend toward the integration of innovative learning methodologies, increasing evidence demonstrates that cadaver dissection remains an irreplaceable part of medical education (Dissabandara et al. 2015; Thompson et al. 2019; Jeyakumar et al. 2020; Huynh et al. 2021; Asante et al. 2021; Orsini et al. 2021; Cullinane and Barry 2023; Pradhan et al. 2024). As a proof of concept, a recent study showed that, in medical students' opinion, replacing physical specimens with augmented reality models would not be beneficial for pathology learning (Moro et al. 2023). Again, many reports have highlighted the facilitation due to cadaveric dissection in classifying human body components and the mapping of the organs and their surface projections (Johnston 2010; Mitchell et al. 2022; Rompolski et al. 2023). In this regard, in his work dating from 25 years ago, Moore claimed the role of human dissection as an educational modality that teaches medical students how to use their hands, helping them to develop touch-based skills used in palpation, percussion, and auscultation (Moore 1998). Another study showed that medical students commonly conveyed that dissection is a valuable educational tool, useful for teaching and learning anatomical knowledge, appreciating structures' spatial relationships and variations, developing teamwork skills, and coping with death/dead bodies (Flack and Nicholson 2018). In fact, besides developing manual skills, getting close to the human body through cadaver dissection can reduce all the emotional impact of an unhealthy and suffering patient, and finally, can introduce students to the reality of death (Older 2004). Therefore, it is important to take into account that dissection should also be considered as an opportunity to strengthen human body familiarization and integration of theory into clinical practice (Lempp 2005).

Within this frame, some authors have emphasized how attending to dissection is fundamental for developing important clinical skills and manual dexterity required of healthcare professionals (Moore 1998; Granger 2004; McLachlan 2004; Slotnick and Hilton 2006). However, compared to the growing interest in students' perceptions and learning outcomes related to medical education using dissection, literature regarding PT conceptions of the human body in relation to experiences with human cadavers in anatomy teaching is extremely scarce (Keim Janssen et al. 2014; Khan et al. 2015; Condo and Justice 2022; Bergen et al. 2023), and so far only a few studies investigate the role of dissection in light of professional PTs' (Latman and Lanier 2001; Rompolski et al. 2023).

Given that anatomy is a core course for healthcare professionals, it is essential to determine whether anatomy education meets PT needs. Since data regarding the usefulness of human cadaver dissection in professional PT education is limited, the present study was designed to investigate the appraisal and attitude toward a dissection course of professionals attending a postgraduate course in "Sports Physiotherapy" at the University of Pisa.

MATERIAL AND METHODS

Subjects

Post-graduate physical therapists (n=56), who attended the course in "Sports Physiotherapy" at the University of Pisa, were involved in the study. The human cadaver dissection course took place at the Institute of Human Anatomy of the University of Bologna, and it was held by senior students at the School of Medicine trained by anatomy teachers.

Three groups of survey participants were identified: the early-career group (licensed less than 1 year before participation in the dissection course), the mid-career group (licensed 1-5 years before dissection), and the late-career group (licensed for more than 5 years before dissection).

The "feedback" survey was sent to the participants via mail at the end of the dissection training. The study was conducted in accordance with the principles of the Declaration of Helsinki. All data were collected anonymously without any possibility to identify the participants. Participants were informed about the nature of the study and their participation was voluntary and without any compensation. Informed consent was obtained from all study participants, who were invited to complete an anonymous questionnaire and encouraged to answer questions openly and honestly. Those who did not want to participate were exempted from completing the survey.

Survey features

The inquiries eligible for the survey were chosen by the authors by matching previous studies presented in the current international literature. Two professional PT were involved in the survey development as expert validators, and their feedback was taken into consideration to draft the final version.

The 30 questions included the following sections: i) demographic data (age, gender, level of education), ii) professional profile (post-qualification experience), iii) anatomy educational approaches (e.g., formal lectures, textbooks, 3D atlases, plastic models), and iv) perceptions of anatomy education including dissection. Furthermore, survey respondents were asked in which year of their physical therapy degree course anatomy was taught, and whether they participated in a dissection laboratory during their anatomy course. They were also surveyed about the perception and relevancy of the dissection to anatomy education and the emotional impact of dissection, pointing out the feelings of anxiety, disgust, and fear.

The participants were asked to give their personal opinion using a 5-point Likert Scale (1 = strongly disagree; 2 = disagree; 3 = neutral; 4 = agree; 5 = strongly agree), which was applied to 15 questions covering the following broader themes: anatomy knowledge, emotional reaction to human dissection, and appraisal of human dissection experience.

Statistical analysis

A Kruskal-Wallis test was used to compare Likert Scale responses from the three groups of survey participants: early, mid, and late-career PT.

The null hypothesis H0 was refused for $p < 0.05$.

RESULTS

The demographic characteristics of all the subjects enrolled in the present study are shown in Table 1. In total, 43 participants filled out the questionnaire, resulting in a completion rate of 76.78 % (43/56). The remaining thirteen dropped out of the study. The respondents' date of birth ranges from 1973 to 2002, thus, it is likely that their answers were not influenced by any social context related to a particular decade.

All responders (100%) attended an anatomy course in the first year of their degree program and the majority of them stated they preferred traditional textbooks

Table 1. Demographic data of study participants.

Demographics	TOT	Early-professional	Mid-professional	Late-professional
Gender				
Female	25.60%	20%	38.46%	20%
Male	74.40%	80%	61.54%	80%
Mean age (years)	25.46±0.78	24.28±0.87	25.23±0.67	32±0.63
Mean experience (years)	2.35±0.37	1±0	2.54±0.18	8.4±0.74
Work setting				
Public hospital	2.33%	0%	7.69%	0%
Private clinic	34.88%	28%	46.15%	40%
Private practice	51.16%	60%	46.15%	20%
Other	11.63%	12%	0	40%

Data are expressed as mean±SEM.

(55.81%), and atlases (30.23%) as the most common learning aids. Of the latter, only 9.30% of respondents adopted 3D atlases as a novel tool for anatomy instruction. Plastic models were used by 6.98% of the responders. Fewer than 10% of respondents stated they used didactic lectures alone.

Before attending the dissection course, 95.34% of respondents agreed that anatomy is taught in a sufficient manner in their physical therapy degree (mean Likert score of 3.21±0.09). Accordingly, less than 5% found it difficult to recognize and locate anatomical structures during dissection (mean Likert score of 2.37±0.10). However, less than 10% have had previous experience with human cadaver dissection during their degree program. At the same time, the majority (93.02%) of the participants stated that they were strongly interested (mean Likert score 4.58±0.12) in attending the dissection course.

With reference to the positive perception of PT on cadaver dissection, 93.02% agreed or strongly agreed that dissection has made anatomy more interesting (mean Likert score of 4.56±0.12), as it provided a three-dimensional perspective of the anatomical structures (mean Likert score of 4.47±0.08). The majority of participants also agreed that the dissection helped strengthen human anatomy knowledge (mean Likert score of 4.28±0.09). Moreover, they considered that participating in cadaver dissection provided clinically relevant anatomical knowledge (mean Likert score of 4.28±0.12) while providing more opportunities to develop professional skills (mean Likert score of 4.35±0.13). Most of them disagree or strongly disagree that dissection can be replaced by educational videos of anatomical models, which indicates high satisfaction with the dissection session (mean Likert score of 2.00±0.21). Besides, respondents also stated that they would have needed more time in the dissection room for more in-depth anatomy knowledge (mean Likert score of 3.19±0.15).

Finally, regarding the negative emotional and physical reactions of the attendants (e.g., anxiety, disgust, discomfort, and fear), only 3 (6.98%) agreed they felt anxiety before dissection (mean Likert score of 1.53±0.13) and 2 participants (4.65%) reported distress when undertaking human dissection. Of note, of the 43 respondents, only 1 perceived the dissection situation as emotionally stressful (mean Likert score of 1.65±0.13). Thus, most of them disagree or strongly disagree about the need for mental preparation before dissection (mean Likert score of 2.33±0.15).

On the contrary, the majority of participants (95.35%) reported that cadaver dissection aroused various positive emotions (e.g., curiosity, interest, satisfaction) and described the experience as interesting and stimulating. In line with this, all respondents stated that they actively participated in the dissection course and remained in the dissecting room all the time (mean Likert score of 4.07±0.12).

Interestingly, when analyzing the data concerning the appraisal of dissection from the different career groups, no significant differences were obtained between groups (Table 2). Indeed, the late-career professional, which implies a more lasting contact with the human body, did not influence the perception and attitude toward cadaver dissection.

Table 2. Opinion of dissection experience of study participants.

Themes	Groups			**p value*
	Early-professional	Mid-professional	Late-professional	
Perception of cadaver dissection				
Do you think your anatomical knowledge was sufficient to take part in the dissection?	3.12±0.10	3.38±0.14	3.2±0.2	0.61
Did you have difficulty locating and/or distinguishing anatomical structures?	2.36±0.11	2.46±0.14	2.4±0.4	*0.78*
Did the dissection help you strengthen your knowledge of human anatomy?	4.16±0.08	4.38±0.18	4.67±0.24	*0.3*
Was the dissection helpful forunderstanding the 3D of the anatomical structures?	4.36±0.09	4.62±0.14	4.6±0.24	*0.45*
Did dissection make learning human anatomy more interesting?	4.44±0.14	4.62±0.18	5±0	*0.38*
Did the dissection experience provide you with clinically relevant anatomical knowledge?	4.12±0.12	4.38±0.21	4.8±0.2	0.2
Attitude towards cadaver dissection				
Did you actively participate in the dissection?	3.96±0.14	4±0.28	4.8±0.12	0.09
Was the dissection emotionally stressful?	1.64±0.13	1.85±0.36	1.2±0.2	0.44
Were you nervous before attending the dissection?	1.60±0.19	1.54±0.18	1.2±0.2	0.67
Do you think it is necessary to prepare students emotionally before carrying out the dissection?	2.16±0.18	2.46±0.27	2.8±0.58	0.42

*95% confidence limits.
Data are expressed as mean±SEM

Finally, overall, all of the respondents believe that cadaver dissection is a unique and privileged experience, and among them, 93.02% would like to attend a dissection course again, if they had the opportunity. In this regard, the participants strongly felt that a dissection course should be offered to undergraduate and professional physiotherapists (mean Likert score of 3.91±0.15).

DISCUSSION

This study aimed to examine early-, mid-, and late-career PT perceptions and attitudes in attending a human cadaver dissection course and how this latter could have a significant impact on their anatomical knowledge, professional identity formation and skills. To the best of our knowledge, this is the first study that investigates perceptions and attitudes of practicing PT in attending a dissection course during a postgraduate course at an Italian university. As highlighted by current literature, much of the research has been focused on undergraduate medical students' experiences of learning gross anatomy from human dissection. At the same time, it is important to point out that, with reference to the Italian educational context, cadaver dissection, as a learning tool, is rarely adopted within Bachelor of Science undergraduate courses in physiotherapy. Contrariwise, dissection courses have globally remained the cornerstone of the medical school curriculum as a potential tool to broaden the range of learning outcomes (Lempp 2005). Indeed, acquiring in-depth knowledge of human anatomy through dissection is considered an academic pillar as it integrates theoretical and practical knowledge, enhances practical skills, and promotes familiarization with dead bodies (Older 2004; McLachlan 2004). Concurrently, previous studies highlight that acquiring manual skills within the dissection room should be crucial for all ongoing clinicians (Prakash et al. 2007). Indeed, the hands-on nature of dissection allows a comprehensive understanding of the three-dimensional aspect of the human body, while contributing to critical relational and topographical knowledge enhancement. Again, undertaking dissection provides the opportunity to observe and experience structures in situ with a texture similar to that of a living body (Webb et al. 2022). In line with this, previous research also indicates that practical training in the dissection of human cadavers can promote an active and engaged experience of how to approach the whole body. In particular, our results showed that all the attendants (100%) consider cadaver dissection a stimulating learning experience and indispensable for understanding the body's structure and function. Within this frame, it appears fundamental to underlie how, although most of the participants' anatomy courses in physical therapy schools did not include anatomy practice, in any case, the dissection course has been regarded as a fundamental tool to improve knowledge of gross anatomy and develop clinical reasoning skills (81.40% of the participants). All the participants also demonstrated a profound interest in learning anatomy because they felt anatomy as crucial to their good and safe clinical practice, thus becoming competent health professionals. Of note, increasing evidence demonstrates that cadaver dissection represents the most productive approach to learning anatomy since it stimulates the investigative approach toward clinical cases and lays the foundations for problem-solving abilities and teamwork skills resembling future collaborative teamwork (Miller et al. 2002). Finally, within the frame of widening scopes of the dissection, it is also important to emphasize how the concentration required during the slow but active sequential process of discovery through dissection represents a unique opportunity for studying gross anatomy (Dissabandara et al. 2015). In this way, the dissection experience allows the students to explore, retain, and recall complex anatomical structures and spatial relations, in a way that other forms of learning anatomy cannot replace.

Interestingly, from the present study, it clearly emerges how the difference in years of practice did not influence the perception towards dissection, and even the late-career participants, who are supposed to be more confident with anatomy structures, stated that the dissection was a great opportunity to enhance the retention of clinically relevant anatomical knowledge and develop clinical skills (100% of the respondents).

Though cadaver dissection has long been considered crucial for learning gross anatomy, it is worth mentioning that some recent studies investigated the feasibility and perceived benefits from students of introducing the use of other "innovative" learning methods and exploring other "cadaverless" anatomy education tools (Singal 2022). This is mainly because some authors reported that students might experience negative attitudes toward dissecting human cadavers (Dempster et al. 2006; Chiou et al. 2021). In particular, most of the negative perceptions towards dissection were related to psychological (stress, anxiety, and emotional trauma) or physical factors, including fear, restlessness, and nausea due to unpleasant smells (Horne et al. 1990; Qamar and Osama 2014; Asante et al. 2021). Other studies reported the potential hazardousness of the chemicals used for cadaver preservation as another dissatisfaction aspect with dissection (Asante et al. 2021). Therefore, some studies suggested that emotional preparation before the beginning of the course or engagement in discussing their experiences and emotions with academic staff after dissection activities could help lower students' anxiety levels (Chiou et al. 2021; Ong et al. 2023). Unlike previous studies, our results demonstrate that most (95.35%) of the surveyed physiotherapists had no negative perceptions of dissection. In detail, among the surveyed PT, only one of the respondents found the dissection of cadavers stressful, while another one felt a little nervous before entering the dissection room. As a proof of concept, 88.37% of them actively participated in the dissection. Consequently, none of them reported the need for emotional support or preparation before starting the dissection.

In any case, it is important to point out that the emotional and physical stress from dissection activities should not be overlooked and more attention should be given to the students facing human cadavers for the first time. Indeed, a stressful experience in the dissection room might determine a negative disposition toward such an experience, which in turn might negatively impact student learning.

Again, the effectiveness of digital support in learning anatomy is still a matter of debate (Miller et al. 2002; Older 2004; Asante et al. 2021). Despite several authors agreeing that computer-simulated models and 3D software are undoubted supports for anatomy education, they present several biases (Older 2004). On the other hand, it is well known that technology tends to dehumanize patient and professional relationships reducing clinician care, and growing rejection of patients towards physicians (Older 2004). Thus, innovative educational tools should be regarded as supplements and not substitutes for human cadaver dissection in the study of gross anatomy. Within this frame, our results demonstrate that all three groups of surveyed participants disagree about the anatomy dissection replacement with video recordings, augmented reality platforms, and 3D virtual models.

In conclusion, although literature regarding physical therapists' conceptions of the human body related to experiences with human cadavers in anatomy teaching is extremely scarce, from the survey reported in the present work it clearly emerges that physical therapists perceive cadaver dissection as highly beneficial to their anatomy education Therefore, greater efforts and attention should be made to include such an invaluable experience of cadaver dissection in the anatomy curricula of physical therapists.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Nittari, G., Savva, D., Traini, E., Tomassoni, D., & Amenta, F. (2024). Effectiveness of facial anatomy cadaver lab training in aesthetic medicine programs. *Italian Journal of Anatomy and Embryology* 128(2):93-99. https://doi.org/10.36253/ijae-15624

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Effectiveness of facial anatomy cadaver lab training in aesthetic medicine programs

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Abstract. Cadaver dissection in post-graduate training programs in aesthetic medicine offers physicians the opportunity of a three-dimensional knowledge of the face morphology, the individual regions, and layers of the face with all neighbouring structures including the main arteries, veins, and nerves. This study has evaluated the effectiveness of this educational intervention, in attendants to the second level vocational Master course in aesthetic medicine and therapeutics co-organized by the universities of Camerino and Turin. Two hundred medicine doctors attending the first year of the course have followed, as a part of the human anatomy course, a 2-day cadaver training on face anatomy at the Hellenic Neurosurgical Research Centre in Athens, Greece. The impact of the program was evaluated by a pre- and post-course multiple choice questions (MCQs) test. Different sets of MCQs were selected for the pre- and post-course test to avoid any recall bias. All 200 participants completed the tests, and their answers were analysed. A significant difference in the number of correct answers and in the scores obtained was found between pre- and post-course MCQs. Answers to questions on muscles of facial expression, muscles of the head, connective tissue and ligaments of the face and blood vessels and nerves of the face were those more correct after the cadaver lab practical course. The results showing a better knowledge of facial anatomical details after a practical cadaver lab suggest the need of including practical cadaver dissection activities in medical training in aesthetic medicine.

Keywords: anatomy, cadaver dissection, aesthetic medicine, anatomy teaching.

INTRODUCTION

A detailed and well-acquired knowledge of anatomy is fundamental for an effective and safe medical practice. Cadaveric dissections represent a way to acquire the organization of the human body through face-to-face visualization of the specific structures directly. It is therefore desirable that courses for medical degree and post-graduate specializations organize specific tutorial activities on cadaver samples different from autopsy and surgical practice modules (Sugand et al. 2010).

A high-quality anatomical background is required for working on complex anatomical structures such as soft tissues of the face which represent a main target for physicians practicing aesthetic medicine. Undertaking aesthetic medicine practice without an on hands training on cadaver may be risky. Learning anatomy just from illustrations and photographs, although it can provide basic knowledge and understanding of anatomy, does not provide the necessary three-dimensional visualization of various structures of the face, their depth and relative proportions. Even advanced computer-assisted anatomy programs cannot provide the sense of the texture of the different layers of human face. Cadaveric dissections offer the opportunity to identify easily and directly the most complex anatomical details including three-dimensional relationships between muscles, arteries, veins, nerves in different regions of the body (Reeves et al. 2004; Aziz et al. 2002; Gregory and Cole, 2002; Mc Garvey et al. 2001; Dinsmore et al. 1999; Marks et al. 1997; Jones 1997). They can also contribute to improving manual skills of operators which are particularly important in a personalized and precision area of medical practice such as aesthetic medicine (Cahill and Carmichael 1985; Ellis 2001).

Intravascular complications associated with the use of injectables in aesthetic medicine procedures are often related with the healthcare professional's lack of enough knowledge and awareness of the at-risk anatomical areas, in particular in the facial region (Levy and Emer, 2012). The importance of anatomy and cadaver training has a decisive impact on the quality of doctors' teaching and consequently on the patient's care. A global consensus on avoiding complications related to the aesthetic/cosmetic procedures also echoed the importance of detailed facial anatomy knowledge to prevent devastating complications (Kumar Ghosh and Kumar 2018; Signorini et al. 2016).

A worldwide increase in the use of botulinum toxin and dermal fillers in skin aesthetics has brought with it a renewed interest in facial anatomy and face dissection practice. A topic with a recognized low priority in aesthetic medicine training in the past. To guarantee a safe and appropriate aesthetic medicine practice, anatomical dissections contribute to ensure optimal clinical results, avoid complications, update surgical and non-surgical techniques, and provide continuing education. (Guttmann et al. 2004; Lempp 2005).

The purpose of the present work was to evaluate the impact of cadaver dissection training on facial anatomy knowledge among doctors practicing aesthetic medicine.

MATERIALS AND METHODS

Participants

The study included 200 physicians who attended a two-year second level Master's program in Aesthetic Medicine and Therapeutics. The course is a vocational Master co-organized by the universities of Camerino and Turin. This is a biennial course requiring 120 (60 per year) ECTS credits for obtaining the Master's diploma. During the first year of the Master as a part of the human anatomy program, the participants attended a 2-day cadaveric dissection anatomy course of the face and neck, on fresh cadavers. Dissections were done at the Hellenic Neurosurgical Research Centre in Athens (Greece), and attendance to the cadaver training was mandatory. The cadaver training in Athens was offered to participants from Greece, Cyprus, Turkey, Bulgaria and the United Arab Emirates for logistic reasons (activity organized closer to their own countries). Participants which were all graduated in medicine and surgery and registered to the corresponding registers for medical practice, were 101 men (50.5%) and 99 women (49.5%) with an average age of 43.8 ± 9.7 years (43.6 ± 10.7 males; 44.0 ± 8.6 females). Thirty percent of them declared to already practice aesthetic medicine before attending the master's course.

Course structure

The cadaver lab course lasted 2 days for 8 hours of activity per day. Day 1 of the course was divided into two sections. The first one consisted in a seminar introducing to the practice of dissection and covering the following topics:

- Structural anatomy of the face;
- Anatomy for botulinum toxin treatments in upper, mid and lower face and neck;
- Anatomy for filler treatments in temple, forehead, orbital areas, middle and lower face;
- Threads (polydioxanone-PDO-monofilament threads and barbed PDO-COG threads) placement in association with anatomic structures.

The second one included video application of all procedures and model applications by the instructors. At the end of the first day a Multiple-Choice Questions test (MCQ) testing the knowledge of the doctors acquired during the first course day was proposed. Day 2 was dedicated to cadaveric dissection practice, with active participation of the students. A fresh cadaveric head was available for every 4-6 physicians. The program included topographic anatomy, layers of the face, fat compart-

Table 1. Structure and contents of the two-day on hands cadaver dissection course.

DAY 1 *Theory: introduction to the practice of dissection.*	DAY 2 *Dissection practice, with active participation of the doctors*
– Anatomy of the Face – layers of the face – fat compartments – muscles and ligaments – arteries, veins, and nerves – bony skeleton	– Face dissection with special attention to: – arteries and veins – fat compartments – muscles and ligaments – bony skeleton
– Anatomy for botulinum toxin injections in upper, mid and lower face and neck	– Botulinum toxin injection practice on face and neck before and after dissection
– Anatomy for filler treatments in temple, forehead, periorbital region, middle and lower face	– Mesotherapy injection practice with needles before and after dissection
– Anatomy for thread application (PDO monofilament and COG)	– Filler injections practice with needle and cannula before and after dissection
– Videos and training on models	– PDO monofilament and COG application before and after dissection
– Test	– Test

ments, muscles, ligaments, main nerves, arteries, and veins as well as bone structures (Table 1). Botulinum toxin injection on the face and neck before and after the dissection, mesotherapy injection with needles before and after the dissection, fillers with needles and cannulas before and after the dissection, PDO monofilament and COG threads before and after dissection were parts of the practical training.

The importance of the dissection after the application of the treatments was that the participants were able to see in real time the depth of their injection, and therefore the effectiveness of their techniques. Day 2 was again concluded with a MCQs test to assess the participants' knowledge and understanding of anatomy after the training. Each session had a logical sequence of teaching activities, which included lectures on the relevant anatomical areas with an emphasis on critical neurovascular and fat compartments, ligaments, and muscles of the upper, mid and lower third of the face and neck (Table 1).

The tests which were different from the first and second day of training consisted in identifying, naming and choosing the correct answer from a list of 6 possibilities on 140 figures for the 7 different face areas as indicated below. Figures were chosen from the volume Clinical Anatomy of the Face (Pessa and Rohrich, 2012).

1 Central Forehead (20 figures)
2 Cheek (25 figures)
3 Eyelids (15 figures)
4 Nose (15 figures).
5 Temporal Fossa (20 figures)
6 Periauricular Region (15 figures)
7 Lips and Chin (30 figures

Moreover, from the text were extrapolated 300 questions/answers per MCQs referring to the following anatomical components of the face: Regions of the head, Facial bones, Muscles of the head, Muscles of facial expression, Subcutaneous compartments of the face, Deep fat compartments of the face, connective tissue and ligaments of the face, Blood vessels and nerves of the face.

Statistical analysis

The data collected were analysed to derive the demographic variables. The answers to the test were processed for identification of variables of position (mean and median) and diffusion (standard deviation) and the standard error.

The pre- and post-course values were then compared using a paired two tailed t-test with a significance level of 0.05. To facilitate comparison, the values were transformed into the percentage of correct answers with respect to the maximum.

RESULTS

Figures 1 and 2 show respectively some practical activities (application of PDO monofilament threats) made by course attendants before and after dissection.

The results of the tests carried out before and after dissection courses are summarized in Table 2. As shown, before and after dissection sessions, responses have a significant difference in correctness. Figure 3

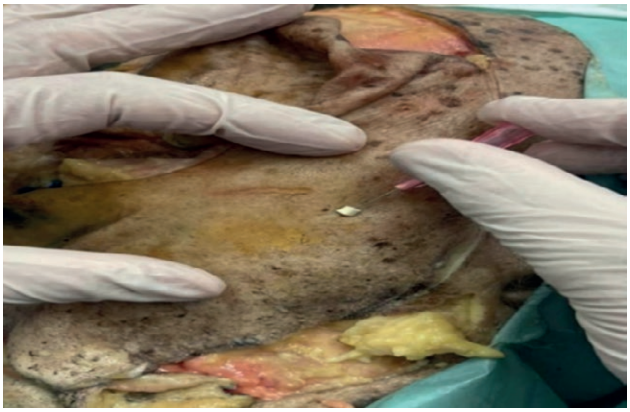

Figure 1. Application of PDO monofilament thread before dissection.

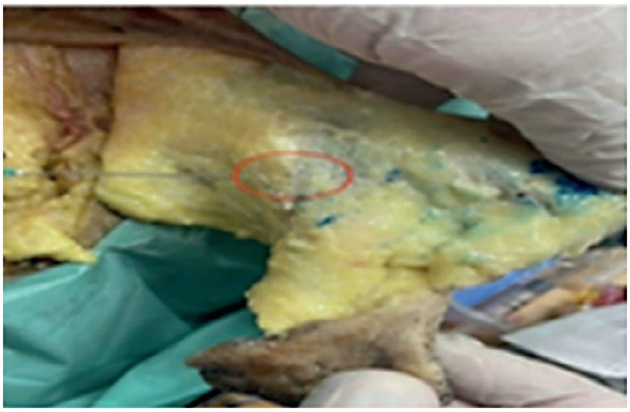

Figure 2. Application and position of PDO monofilament thread after dissection.

shows the percentages of scores obtained in pre- and post-dissection tests referred to the maximum possible score. Knowledge achieved by attendants on different topics of the test after cadaver training involved in the order the muscles of facial expression, muscles of the head, connective tissue and ligaments of the face and blood vessels and nerves of the face. No sex or age-related differences among attendants was noticeable in terms of scores obtained at the second versus the first test (data not shown).

DISCUSSION

Despite the commonly recognized relevance of cadaver practice in medical training, nowadays teaching of anatomy using the cadaver is significantly decreased due to the adoption of integrated medical curricula

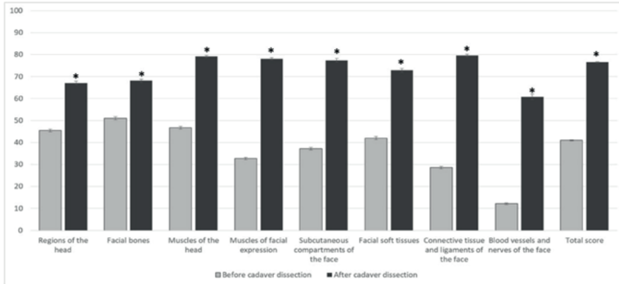

Figure 3. Percentages of scores obtained in the test referred to the maximum score obtainable, before and after the dissection course. Data are the mean ± S.E.M. *= $p<0.05$ vs. Pre-course values.

around the world, costs, the time required to perform the actual dissection modules and the enrolment of non-medical professionals as professors of human anatomy (Drake et al.,2009; Kumar and Rahman.,2017). A decrease in the quality of university education in anatomy is often reported (Monkhouse, 1992; Heylings, 2002; Older, 2004; Waterston and Stewart, 2005; Warner and Rizzolo, 2006; Turney, 2007). The cause of it can be attributed mainly to the reduction of time and contents of macroscopic anatomy courses (Moxham and Plaisant 2007; Drake et al. 2009) and to the growing gap between pre-clinical and clinical programs of medicine courses (Hirt et al. 2010). A relevant number of health professionals fails to remember a considerable amount of anatomical knowledge in the transition between preclinical teaching and the clinical phase of their education (Custers 2010; Hirt et al.,2010).

The rapid rise of the interest in cosmetic/aesthetic surgery/medicine minimally invasive procedures and the increasing efficacy and safety of office-based aesthetic procedures in reducing the signs of facial aging, is attracting patients towards non-surgical facial cosmetic procedures, compared to traditional aesthetic surgical approaches (De Aquino et al. 2013). A better understanding of facial anatomy through adequate training may improve the success of treatment and to obtain optimal clinical results (Shaw et al. 2011). Another potential added value of cadaveric dissection is that it requires the direct involvement of the learner, as opposed to other "passive" teaching methods. An evaluation of six different teaching modalities (frontal lectures, small group teaching, dissection, small group teaching with the aid of computer tools, preparation of thematic in-depth studies, and tutorials for dissection) of the Preparatory Diploma in Biomedical Research has demonstrated that the most effective results come from learning through the dissection modules (Voiglio et al. 2002).

Table 2. Scores obtained in the pre- and post-dissection session test by the attendants to the Master in Aesthetic Medicine and Therapeutics.

Topics of the test (maximum score reachable)	Before dissection Session	After dissection Session	Differences	Significance (p<0.05)
Regions of the head (40)	16.38 ± 4.14	24.16 ± 4.59	7.78 ± 4.66	9.87 E-89
Facial bones (50)	31.66 ± 8.01	42.19 ± 8.45	10.54 ± 10.10	7.39 E-50
Muscles of the head (60)	30.83 ± 7.45	52.24 ± 5.60	21.41 ± 8.96	7.52 E-126
Muscles of facial expression (50)	17.01 ± 4.53	40.62 ± 5.07	23.61 ± 6.77	0
Subcutaneous compartments of the face (20)	7.44 ± 2.32	15.48 ± 3.19	8.03 ± 3.98	1.26 E-107
Deep fat compartments of the face (30)	10.29 ± 3.50	28.64 ± 3.79	18.35 ± 5.19	0
Connective tissue and ligaments of the face (20)	6.30 ± 1.70	10.92 ± 2.46	4.62 ± 3.00	4.34 E-81
Blood vessels and nerves of the face (30)	3.03 ± 1.41	15.20 ± 5.69	12.17 ± 5.85	9.99 E-111
Total score (300)	122.95 ± 13.87	229.45 ± 18.65	106.51 ±20.87	0

Values are the means ± Standard deviation.

In view of the above consideration, as a part of the program of human anatomy of the vocational Master in aesthetic medicine and therapeutics we have developed an original hands-on educational training two-day course based exclusively on cadaver dissection. The target of the dissection course are attendants to the first year of the Master and the course is repeated every year. In 4 years of experience the course was followed by a total of 200 physicians.

Measuring the quality of training and knowledge has been debated. There is no universally agreed measure of learning. Different learning methods are currently applied by various institutions such as lectures, teaching in small groups, dissection, teaching in small groups with the help of IT tools, and preparing thematic in-depth studies, which do not always produce equivalent results (Warner and Rizzolo, 2006; Turney, 2007). Multiple-choice questions allow testing both memory and recognition (Arzi et al. 1986). In our work at the end of the first and of the second day of the course, a 1-hour formal test was administered with 840 MCQs divided into the above thematic areas. A score of 1 was assigned to each correct answer. A score of 0 was assigned to each incorrect or empty answer. A similar test was repeated at the end of the dissection course of the second day.

Overall, this study has demonstrated that the cadaver dissection course contributes to a significant improvement of the knowledge of facial anatomy to professionals involved in aesthetic medicine practice. The goal of the course was to provide trainees with in-depth facial anatomical knowledge for increasing safety and efficacy of injectable treatments, to minimize complications and to ensure the best results for the patient. We can therefore conclude that cadaver dissection is a valuable educational tool for understanding facial anatomy to guarantee the necessary safe outcome for patients after any non-surgical injectable aesthetic treatment (Özcan et al. 2015). The course contributed to develop a significant improvement of the anatomical knowledge of the different face areas as shown by the results of the pre and post training dissection tests. The most relevant results were the improvements in knowledge of the anatomy of muscles of facial expression (23.6%), muscles of the head (21.4%) connective tissue and ligaments of the face (18.3 %) and blood vessels and nerves of the face (12.1 %).

The results of this study show that cadaver dissection familiarizes physicians not only with anatomical details, but also with anatomical variations and an appreciation for particularly exposed structures, such as vessels and fat compartments, which could potentially be damaged or injured during cosmetic treatments (Turney, 2007; Dissabandara et al. 2015). Our results are consistent with the conclusions of another study reporting an advantage of cadaveric dissection over more recent techniques, concluding that "*anatomical knowledge is too important for future physicians to leave its teaching to the educational fashion of the day* " (Winkelmann, 2007).

Despite the important contribution of new technologies, there still exist areas of medical practice in which teaching and training on cadaver samples have a decisive impact on the quality of doctors' techniques and, consequently, on the quality of health care for the patients. The direct experience on cadaver specimens still seems to remain irreplaceable, even though it can take advantage of the integration with multimedia methods and increasingly advanced technologies (Kumar Ghosh and Kumar, 2018).

LIMITATIONS

This study presents several limitations. No control group was used while carrying out this study. However, in this work, a pre-seminar dissection test was created, and a post-seminar dissection test was performed, where the pre-seminar test results are serving as a control group. Our hypothesis was the significance of cadaver dissection in expanding the knowledge of physicians which is supported through our study (Regehr 2002; Eliopoulos et al. 2004). No repeated measurements were taken, and it is impossible to evaluate the long-term preservation of the acquired knowledge. However, we made sure that the procedures were thorough, demonstrable, and repeatable.

CONCLUSIONS

Given the capabilities of modern diagnostics techniques, knowledge of the anatomical variants found on the cadaveric specimens can help the improvement of anatomical knowledge, understanding and confidence of aesthetic doctors and consequently the safety of the patient. The study of specific anatomical areas has regained relevance since specific functional and pathophysiological problems have been identified, such as the need for more feedback in "normal" anatomy. Anyone who works on a patient must acquire knowledge of the real body and technical-manual skills to achieve a degree of ability and autonomy aimed at the successful outcome of the procedures used and to ensure patient safety first.

ACKNOWLEDGMENTS

This work was realized in the frame of second level Master course in Aesthetic Medicine and Therapeutics, supported by an institutional grant of the University of Camerino.

FUNDING INFORMATION

This paper was supported by an institutional grant of the University of Camerino. The content of the paper is the sole responsibility of the authors and can under no circumstances be regarded as reflecting the position of the supporting institutions.

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IJAE
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Guidolin, D., Tortorella, C., Anderlini, D., Marcoli, M., & Agnati, L.F. (2024). The brain as a "hyper-network": impact on neurophysiology and neuropharmacology. *Italian Journal of Anatomy and Embryology* 128(2): 101-111. https://doi.org/10.36253/ijae-15710

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

The brain as a "hyper-network": impact on neurophysiology and neuropharmacology

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Abstract. Neuronal network architecture plays a crucial role as the structural substrate for the brain functions. Increasing evidence, however, indicates that, beside neural networks, to fully understand brain complex integrative actions glial cells and the diffusion of signaling substances in the network of extracellular fluid channels should also be considered. To account for this more complex architecture it has been proposed that all these networks are assembled into a so-called brain hyper-network, having as fundamental components the multi-partite synapses involving not only neurons, but also regulated by the astrocyte networks and fine-tuned by microglia and by pervasive signals diffusing in the interstitial channels of the extracellular matrix. The main features of this view of the central nervous system organization are here discussed. This complex network architecture can be of particular interest for neurophysiology, since it may represent a suitable structural counterpart of physiological mechanisms allowing goal-directed behavior. Furthermore, a model of brain organization integrating the activity of different CNS components may assist in the identification of new possible targets for the pharmacological treatment of CNS diseases. These aspects are also briefly discussed.

Keywords: multi-partite synapse, intercellular communication, astrocyte networks, extracellular matrix, receptor complexes.

INTRODUCTION

In the last decades a large body of new evidence on central nervous system (CNS) structure and function has been acquired by morphological investigations based on a combination of different approaches, such as chemical neuroanatomy methods (histology, histochemistry, and immunocytochemistry), new techniques in microscopy (e.g. confocal or atomic force microscopy), and brain imaging technologies. A next key step was the possibility of quantitative analysis of the obtained images, mainly achieved by the application of computer-assisted image analysis methods (Guidolin et al., 2022). This combination of different approaches led to a view of the CNS as a huge network of cells, regions, and systems in which intercellular communication processes virtually determine all aspects of the integrative function performed (see Agnati et al., 2023 for a discussion).

The network architecture plays a key role as a structural substrate for the CNS functions as indicated by the increasing interest for the connectomics, the comprehensive study of all aspects of neuronal connectivity (Lichtmann & Denk, 2011; Sporns, 2012). This field, representing a great scientific challenge in neuroanatomy, has developed very rapidly in the last years, allowing the characterization of the anatomic connections of large brain regions and functional neural subcircuits (Hagmann et al., 2007; Gong et al., 2009; Briggman et al., 2011; Van Essen et al., 2012; Wang & He, 2024; see also https://www.humanconnectome.org/).

In this respect, however, it is noted that although the fundamental anatomical substrate of CNS function are neural networks resulting from synaptic contacts among neurons, they do not deal exhaustively with the issue. About 40 years ago, several authors (see Hökfelt et al., 1986) demonstrated that one neuron could synthesize more than one neurotransmitter and a single postsynaptic site may express different types and subtypes of receptors for a given transmitter, with each receptor controlling a different decoding mechanism. Thus, a synapse becomes endowed with multiple communication/transmission lines. Furthermore, our group and other groups (see Guidolin et al., 2017 for a review) provided new data on the communication modes in the CNS that, while not dismissing the fundamental relevance of synaptic contacts, allowed to identify (Agnati et al., 1986) the existence of two main modes of intercellular communication in the CNS, generically called wiring transmission (WT: point-to-point communication as in the synaptic transmission) and volume transmission (VT: communication by diffusion in the extracellular/cerebrospinal fluid).

In the 1990s (see Araque et al., 2001), a broadened view on the cellular organization of the CNS was also provided with the demonstration that neurotransmitters can elevate astrocytic calcium levels as the result of the release of calcium from internal stores. This wave of intracellular calcium elevation was shown to propagate through gap junctions (Allen and Barres, 2005) between astrocytes for hundreds of micrometers (Cornell Bell et al., 1990), indicating the existence of astrocytic networks. In the involved astrocytes the calcium signal stimulates the release of gliotransmitters (such as D-serine, ATP, glutamate), leading to a direct regulation of ongoing synaptic activity (Fellin and Carmignoto, 2004). In this context, of particular interest have been ultrastructural investigations (Ventura and Harris, 1999) indicating that a high number of thin filopodia- and lamellipodia-like astrocytic processes (called PAP) can contact and enwrap synapses, the sites of neuronal communication, sometimes completely encapsulating them. Thus, the function and efficacy of synaptic transmission are determined not only by the composition and activity of pre- and postsynaptic components but also by the features of the PAP that enwrap the synapse. This evidence led to the proposal of the concept of "tripartite synapse" (Araque et al., 1999), providing a link between neural and astrocytic networks.

Although most of the available histochemical studies focused mainly on nerve cells, extracellular molecular networks have been revealed to play significant roles in the functional and structural organization of the brain (see Agnati et al.,2006 for a review). Extracellular matrix (ECM) components are synthesized by both neurons and astrocytes and are involved in the formation, maintenance, and function of synapses in the CNS (Dzyubenko et al. 2016; Rauch, 2004). Furthermore, they have a key role in the VT intercellular communication, since this communication mode is based on the diffusion of neuroactive substances in the brain extracellular space and their binding to extrasynaptic high-affinity receptors on neurons or glia (Nicholson and Sykova 1998; Agnati and Fuxe, 2000; Marcoli, 2015).

The possibility to integrate the abovementioned findings in a new overall view has been explored. In particular, the conceptual model of the brain as a hypernetwork (BHN, including neural networks, glial networks and ECM as components) has been proposed by our group (Agnati et al., 2006; 2018; 2023; Guidolin et al., 2017). It is schematically illustrated in Fig. 1 and its main features are discussed below. This complex network architecture can be of particular interest for neurophysiology, since it may represent a suitable structural counterpart of physiological mechanisms allowing goal-directed behavior, as those addressed in the framework of the theory of functional systems (see Sudakov, 1997). Furthermore, a model of brain organization integrating the activity of different CNS components may also assist in the identification of new possible targets for the pharmacological treatment of CNS diseases (Marcoli et al., 2023). A brief discussion of these aspects will be provided in the sections that follow.

THE BRAIN AS A HYPER-NETWORK

The architecture of the CNS extends over a range of up to five orders of magnitude of scales: from microns for cell structures at one end to centimeters for inter-areal neuronal connections at the other. As far as the neuronal networks are concerned, evidence has been provided that the connections between different brain areas exhibit an organization called "small- world networks" (Watts and Strogatz, 1998; Liao et al., 2017), forming clusters of nearby areas with short links, which, in turn, have long-range connections to other clusters (Sporns and Zwi, 2004; Stam and Reijneveld, 2007).

Based on these observations, CNS tissue can be described as composed of a set of compartments or "functional modules" (FM; Agnati and Fuxe, 1984; Robertson, 2013) delimited by plastic boundaries. Thus, as illustrated in Fig. 1A, FM were considered the basic organizational level of the BHN architecture proposed for the CNS (Agnati et al., 2018). Typical examples of FM are provided by the human cerebral cortex, where 100 or more anatomical regions can be defined (Wig et al., 2011).

Available anatomical findings on these cortical areas also provide suggestions concerning the internal architecture of each FM. They, indeed, appear organized in cortical columns (Lorente de Nò, 1938; Mountcastle et al., 1957), cylinders composed of vertical chains of cells crossing all cortical layers. As stated by Rakic (Rakic, 2008), cortical columns can be considered functional units subserving a set of common static and dynamic cortical operations. A similar organization appears to characterize thalamus (Boeken et al., 2023), hippocampus (Caroni, 2015), basal ganglia (DeLong and Wichmann, 2009) and cerebellum (Leggio and Olivito, 2018). Within each cortical column (diameter in the range of 300–500 μm) minicolumns (diameter of about 50 μm) can be distinguished (Pethers and Sethares, 1996). According to Rinkus (Rinkus, 2010) they have a generic functionality, which only becomes clear when seen in the context of the function of the higher level, subsuming unit, the cortical column. Thus, a FM can be modeled (Agnati et al., 2018;2023) as formed by microcircuits (Fig. 1B) in which neurons and glial cells (mainly astrocytes) are organized into specific patterns to carry out processing activities (Shepherd, 2011).

Concerning the intercellular communication within FM and among FM, WT processes play a key role, being the involved neurons connected by means of synaptic contacts, and astrocytes through gap junctions (mediating the propagation of calcium signals between them). The intercellular communication between the cells in the CNS, however, is not limited to specific districts of these cells, such as synaptic regions or gap junctions (where the involved cells are in contiguity), but it also includes processes of VT, based on the release of signaling molecules and their diffusion in the extra-cellular space (see Guidolin et al., 2017 for a review) for a distance greater than the synaptic cleft. Typical chemical signals diffused by VT (see Guidolin et al., 2017; Agnati et al., 2023) include neurotransmitters, neuromodulators, growth factors, hormones, ions (e.g., Ca2+ ions) and gases (e.g., NO, CO_2, CO). This communication mode uses the several often spatially divergent tortuous channels made by the clefts (about 20 nm in diameter) between cells and filled with extracellular fluid and extracellular matrix (Chen and Nicholson, 2000). VT, therefore, is characterized by a very high divergence, since one source usually can send signals to a great many targets, including not only neurons and astrocytes but also other types of cells in the CNS, such as microglial cells (Färber and Kettenmann, 2005). In this respect, of potential interest is also the suggested possibility that electric fields produced by neuronal activity (Anastassiou and Koch, 2015) and magnetic fields associated with Ca^{2+} transients in astrocytes (Martinez-Banaclocha, 2017), if strong enough and/or positioned precisely, could influence the electrical excitability of neighboring neurons through a process called ephaptic coupling (Hales and Pockett 2014; Scholkmann 2015; Agnati et al., 2018). This multifaceted pattern of signaling leads to the formation of "complex cellular networks," exchanging signals in a certain volume of brain tissue and, due to this cross talk, integrating their activity (Agnati and Fuxe, 2000).

In this context, of particular interest is the role played by astrocytes at the level of the "tripartite" synapses (Araque et al., 1999), the structures providing a direct link between neural and astrocytic networks. As a response to synaptic activity (Heller and Rusakov 2015; Ghézali et al. 2016), indeed, astrocytes associated to these structures are able to rapidly (time scale of minutes) restructure their peri-synaptic processes modifying the coverage of the synaptic contacts (Reichenbach et al. 2010; Bernardinelli et al. 2014). Such a sophisticated control of the PAP's plasticity, therefore, could allow moving from a high privacy of the synaptic transmission (close enwrap of the synapse) to a more or less broad opening of the enwrapping. This would lead to diffusion by VT of signaling molecules also to neighboring astrocytes, neurons and other glial cells (Grillner and Graybel, 2006; Dallerac et al., 2013; Marcoli et al., 2015; Agnati et al., 2018; Semyanov, 2021). To account for this more complex network of signaling, more recently the concept of "multi-partite synapse", whose dynamics involves not only neuronal synapses and astrocytes but also the extracellular matrix and microglial cells, has been proposed (Agnati et al., 2018; 2023; Aramideh et al., 2021). It is schematically illustrated in Fig. 1C.

It is well known that at the cellular level membrane receptors represent the key mechanism to decode the incoming signals. Experimental findings also showed that these elements of the cell decoding apparatus can be transferred to another cell via the exosome pathway (see Guidolin et al., 2023a for a review), enabling the target cell to transiently acquire the capability to decode signals for which it does not express the pertinent receptors. This process (called "roamer-type" of VT) represents a mechanism of plasticity for intercellular communication. In the 1980s, *in vitro* and *in vivo* experiments by Agnati, Fuxe and collaborators (Agnati et al., 1980; Fuxe et al., 1983) provided indirect evidence that a major class of cell receptors (the G protein-coupled receptors, GPCR), able to signal as monomers, can also establish structural receptor-receptor interactions (RRI), leading to the formation of receptor complexes (dimers or high order oligomers) at the cell membrane (see Fuxe et al., 2007 for historical details). In the years that followed, several groups (see Guidolin et al., 2022 for references) provided direct evidence for the existence of this molecular organization by exploiting a set of experimental techniques able to detect the spatial proximity of protein molecules (Trifilieff et al., 2011; Petazzi et al., 2020). The basic molecular mechanism characterizing the receptor assemblies are allosteric interactions (Changeux and Christopoulos, 2017), allowing the transfer of the energy associated with conformational or dynamic changes at some site of a protein to other sites, that will change their conformational or dynamical features accordingly. The resulting collective dynamics of these supramolecular structures allows the integration of different incoming signals reaching the plasma membrane to initiate specific patterns of signal transduction (Fuxe and Borroto-Escuela, 2016). Interestingly, bioinformatics approaches indicated that the dynamics of receptor complexes can be described by suitable networks models (Guidolin et al., 2007) suggesting that the connections between cells are themselves networks (molecular networks).

As illustrated in Fig. 1D, according to the above discussed features the BHN model appears to display a hierarchical or nested ("russian doll") architecture (Agnati et al., 2018; Guidolin et al., 2023b), providing a unified view of the different spatial scales (from the macro-scale of brain areas and FM, to the nano-scale of the molecular networks) characterizing the brain network organization. This view, supported by experimental findings focused on neuronal networks (see Sporns et al., 2005), can be discussed in the frame of Jacob's proposal (Jacob, 1977) on evolution working not as an engineer but as a tinkerer. Jacob claims that evolution tinkers together contraptions in a natural selection process that acts by adding direction to changes, orienting chance, and slowly and progressively producing more complex structures.

ANATOMICAL ARCHITECTURE AND NEUROPHYSIOLOGY

The search for understanding the role of CNS in the control of physiological functions has been classically subdivided into two disciplines, neuroanatomy and neurophysiology, which analyze from complementary perspectives the structural and functional properties of the CNS. As evidenced by the work of the neurobiological pioneer Santiago Ramon y Cajal (1852-1934), however, this historical dichotomy was never absolute (Borst and Leibold, 2023). He, indeed, always studied anatomic structures with reference to functional insights and modern functional imaging techniques (Friston, 2009) make the distinction quite blurred.

For centuries physiology based its view of the role of CNS function on the 'reflex principle' (see Sudakov, 1997 for a discussion), assigning a leading role to sensory stimuli, whose propagation along nerve fibers, from receptors to specific nerve centers, would allow the production of a reflected action as a final stage. According to this view, brain areas should be highly selective and exhibit considerable specialization, each responding to a set of inputs and contributing primarily to a single cognitive domain. Reflex theory, however, failed to provide a satisfactory explanation of many complex phenomena (Sudakov, 1997). This especially concerns goal-directed behavior. Reflex theory, indeed, had a difficulty in explaining why living organisms are so skilled in inventing means to correct their behavioral errors, and why their activity is not limited to responses to external stimuli but goes on until a certain vitally important result is achieved.

In the early 1940s an alternative view, called 'theory of functional systems' (TFS), was proposed (Anokhin, 1937). According to this view, some organism need is the dominant factor driving the organization of brain activities to achieve a result. This occurs through a flow of information (sensory, contextual, motivational, mnemonic) reaching brain areas, leading to their self-organization in order to define a goal and trigger possible goal-directed actions (Müller et al., 2021). When the goal is being set, however, we have the goal but not the result (Vityaev and Demin, 2018). Thus, the brain organization is pro-

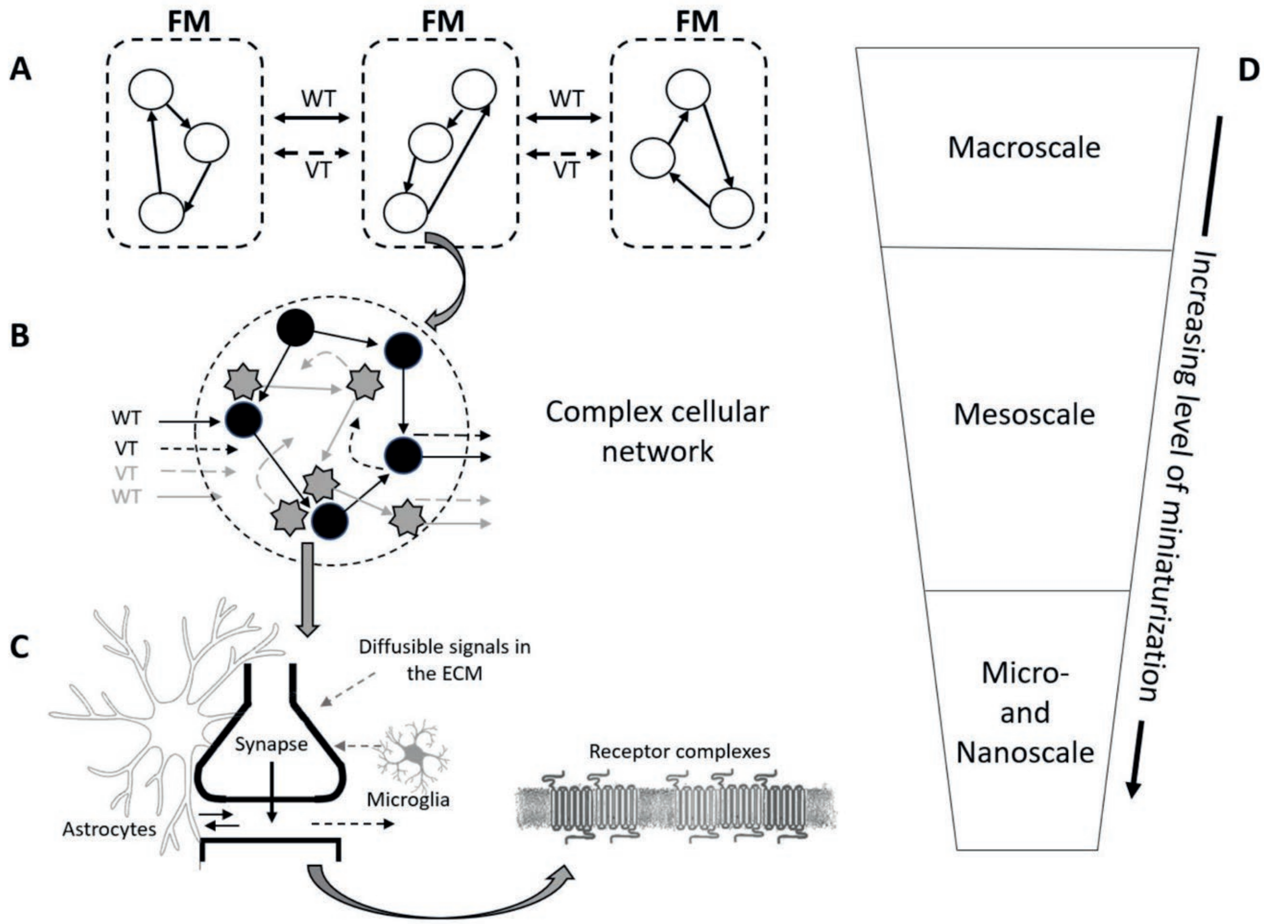

Fig. 1. Schematic illustration of the BHN view of the CNS organization. **A.** CNS tissue can be described as a network of FM linked by WT (solid arrows) and VT (dashed arrows) signaling pathways. **B.** Each FM can be modeled as formed by microcircuits involving neurons (in black) and glial cells (in grey) communicating by WT (neurons and astrocytes) and/or VT. **C.** Crucial components of this organization, are multi-partite synapses, whose dynamics involves not only neuronal synapses and astrocytes but also the extracellular matrix and microglial cells. Furthermore, at the cell membrane receptor proteins can form quaternary structures (receptor complexes). The collective dynamics of these supramolecular structures allows the integration of different incoming signals reaching the plasma membrane to initiate specific patterns of signal transduction. **D.** According to the BHN view the CNS tissue exhibit a hierarchical organization with networks of increasing miniaturization nested within each other.

gressively adjusted depending on mechanisms of feedback on the degree of usefulness or value of the currently obtained result (Anokhin, 1974; Vityaev and Demin, 2018). The brain functional organization, therefore, is not seen as a rigid design, but rather as a constantly changing dynamic structure. Only the elements that lead to the desired result are selected, and this selection is in a constant flux and evolution (Müller et al., 2021).

In this respect, being composed of a network of FM, the BHN model of brain architecture fits with this physiological view of CNS function and provides support to that view. The model, indeed, is consistent with findings from brain imaging studies showing that most regions of the brain appear to be activated by multiple tasks across diverse task categories (Anderson et al., 2010). As an example, studies on the Broca's area (see Poldrack, 2006), showed that the current notion of the Broca's area as a specific "language" region is weak, since it was more frequently activated by non-language tasks than by language-related ones. These findings suggest that the brain achieves its variety of functions by putting the same regions together in different patterns of functional cooperation.

The possibility of different patterns of activity is a typical feature of the dynamics of systems characterized by a network architecture. In other words, when started from an initial configuration generated by external inputs, a network system can rapidly converge to one of a number of temporary equilibrium configurations or ‘attractors’ (Wuensche and Lesser, 1992; Guidolin et al., 2007; Pereira and Brunel, 2022; Ashwin et al., 2024). Thus, the BHN model of CNS architecture may also represent a suitable structural counterpart of this physiological process.

In this respect, a further intriguing aspect emerges when the hierarchical architecture of CNS suggested by the BHN view is considered. Physiological processes, indeed, are in general controlled at multiple cellular levels and neural circuits indicating a hierarchical functional organization (Nederbragt, 1997) allowing an increasing level of integration of the incoming information. Reported examples include the maintenance of homeostasis (Stevenson, 2024) and visual perception (Gilbert, 2013; Gämänut and Shimaoka, 2021; Lima et al., 2023).

A last point, however, has to be emphasized. As mentioned before, a complex flow of information is physiologically important to trigger possible goal-directed actions. In this respect, the hypernetwork architecture can be of specific interest. This architecture, indeed, considers a spectrum of signals reaching brain areas not limited to neuronal synaptic signaling, but also involving signals between astrocytes, between neurons and glial cells, as well as signals (such as growth factors, hormones, ions, and gases) reaching the nerve cells by VT pathways. Therefore, the use by brain areas of contextual information to define their functional organization is intrinsically present in the BHN view of CNS architecture.

ANATOMICAL ARCHITECTURE AND NEUROPHARMACOLOGY

A critical aspect of drug development in the therapy of neuropsychiatric disorders is the so-called “target problem” (TP), namely the selection of a proper target not simply based on the etiopathological classification of symptoms but rather on the detection of the supposed structural and/or functional brain alterations (see Marcoli et al., 2023 for a recent review).

In neuropharmacology the classical view of neuronal synapse is still the most followed reference framework on which drug discovery and development are based. Early findings, indeed, suggested that by acting at the synaptic receptor level, marked changes in integrative brain functions could be achieved (Kebabian and Greengard, 1971; Snyder, 2011; Giessing and Thiel, 2012). However, frequent failure of drugs in drug development and/or drug side effects, especially during chronic treatments, indicated that the TP was not well resolved by this direct approach (see, for instance, (Lipton, 2005) for N-methyl-D-aspartate receptor as drug target). A step forward was the characterization at synaptic level of iso-receptors (i.e. of receptor subtypes for the same neurotransmitter), offering the possibility of acting on recognition/decoding components of synaptic transmission capable of triggering some peculiar responses at synaptic level (see Snyder, 1984). Again, however, TP was not fully solved with the more selective drugs targeting isoreceptors. Indeed, although less severe than previously observed, side effects and/or treatment failures have been reported (Miller, 2010; Carhart-Harris and Nutt, 2017; Charvin et al., 2018).

The more comprehensive view of synapses as multipartite structures, as suggested by the BHN model of CNS organization (Agnati et al., 2023), may significantly expand the range of possibilities to addressing the TP (Marcoli et al., 2023). They, indeed, don’t involve only neurons, but are also regulated by the astrocyte networks and fine-tuned by microglia (Miyamoto et al., 2013; Crapser et al., 2021) and by pervasive signals diffusing in the interstitial channels of the extracellular matrix.

In this context, membrane receptor complexes in neurons and glial cells have been proposed as key integrators, capable of converting multiple extracellular signals into appropriate cellular biochemical responses (see (Guidolin et al., 2021;2022;2024) for reviews). RRI, therefore, may provide new opportunities to optimize existing pharmacological treatments or to develop completely new pharmacological strategies. In this respect, the search for receptor heteromers’ selective compounds would be of key importance to fully exploit their properties. At least three approaches could be followed to achieve this goal. The first, and presently most studied (see Guidolin et al., 2020), is based on the fact that, due to a different pattern of allosteric RRI, the conformational state of a given protomer may change according to the type of complex in which it is involved (Fuxe et al., 2013). Thus, the pharmacology of some agonists/antagonists of a given protomer in terms of affinity and efficacy may show substantial differences among various types of receptor complexes. A second approach to identify receptor complex selective compounds is based on the possibility that, when the complex forms, the quaternary structure could display novel specific allosteric sites suitable for the binding of some modulators (Cervetto et al., 2008). The use of bivalent ligands constitutes a third possible approach for targeting receptor heteromers (see (Hiller et al., 2013) for a review). A bivalent ligand consists of two pharmacophoric entities linked by an appropriate spacer. In this way, it should be possible to target GPCR heteromers by adequate, potent, and receptor complex-selective ligands. (see (Daniels et al., 2005) for examples). These research efforts are still in their experimental phase. Some significant results, however, have been obtained, as exemplified by the adenosine A_{2A} receptor antagonist istradefylline (targeting the heterodimer between the A_{2A} receptor and the dopamine D_2 receptor), recently approved in the United States as an adjunctive treatment in Parkinson's disease (Chen and Cunha, 2020).

CONCLUDING REMARKS

As indicated by the rapid increase of studies addressing connectomics, the anatomical mapping of the relationship among CNS components can represent a significant advance to reach a deeper level of understanding of CNS functions. In fact, the integrative actions of networks in which functions emerge from sets of elementary units (nodes), linked by connections and bound together dynamically (Bullmore and Sporns, 2012), are probably the process allowing the brain to accomplish its activity. As pointed out by Sporns (Sporns, 2013), the emphasis on structure is important because anatomically determined connections among CNS elements embody a large but finite set of relations that (at least in principle) can be objectively characterized in terms of their geometrical and biophysical features.

To date, the characterization of the connectivity between neurons at a macroscale level (Gong et al., 2009) has been the major focus of the research effort in connectomics, allowing the demonstration of several topological features of the adult human neuronal networks (see Stam, 2010). Experimental and theoretical limitations of the present approach, however, exist and should be carefully considered. The major limitation rely to the assumption that all functionality of CNS could be derived once the complete pattern of connections between neurons has been recorded (see Sporns, 2013, for a critical analysis).

In this respect a particular aspect deserves consideration. It refers to the increasing evidence indicating that synaptic transmission is significantly complemented by other cell types (Araque et al., 1999; Miyamoto et al., 2013) communicating via two modes of connection, WT and VT (which are not mutually exclusive), and by a pattern of diffusing signals reaching the cells through the extracellular space (Agnati and Fuxe, 2000; Marcoli et al., 2015). The view of the CNS organization as a hyper-network tries to account for this more complex architecture to reach a deeper insight into the relationship between brain structure and function with potentially relevant implications of this enlarged view on neurophysiology and neuropathology (see Guidolin et al., 2017).

Obviously, at present, it is impossible to give detailed representations of the BHN. The possibility however exists to identify the brain areas where are mainly concentrated the supposed crucial components of this organization, namely the multi-partite synapses (where the dynamic reassembling of the different brain networks information handling processes appears to occur) and to consider these areas as nodes for an analysis of the BHN (see Robertson, 2013). A research effort in this direction could also complement imaging connectomics and provide a more complete drawing of the connectome plasticity in different functional conditions. Furthermore, it can be suggested that investigations on the functional plasticity of multi-partite synapses can be the background for a new understanding and perhaps a new modelling of brain integrative actions.

In the last 20 years, a significant research interest has also been focused on the nano-scale level of the BHN, namely on molecular networks at the cell membrane of neurons and glial cells (see Guidolin et al., 2021), and in particular on receptor complexes. They, indeed, may provide new opportunities to optimize existing pharmacological treatments or to develop completely new pharmacological strategies. In this context, a topic of possible development would also be the identification of pharmacological tools separately targeting synaptic and extrasynaptic receptors (Hoestgaard-Jensen et al., 2013) in order to design strategies to rebalance WT and VT. In the neuropharmacological field, finally, an important direction of future research is certainly targeting glial cells as a strategy to treat neurological disorders (Cervetto et al., 2023). As suggested by the BHN view, indeed, the intimate association of glia with neurons is at the basis of increasing evidence that metabolic perturbations of glial cells may alter neuron–glial interactions, potentiating the underlying pathology of many neurological diseases (Afridi et al., 2020). The mechanism driving the circumstantial activation of glial phenotypes, however, is just starting to unravel, and future studies should open new perspectives.

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OPEN ACCESS

Citation: Benagiano, V., & Rizzi, A. (2024). Autonomic functions of the cerebellum: Anatomical bases and clinical implications. *Italian Journal of Anatomy and Embryology* 128(2): 113-123. https://doi.org/10.36253/ijae-15756

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Autonomic functions of the cerebellum: Anatomical bases and clinical implications

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Abstract. Traditionally, the cerebellum is viewed as a center for integrating vestibular and general proprioceptive sensory, enabling the processing of somatic motor responses essential for maintaining balance and posture. Moreover, the cerebellum regulates higher motor functions of the neocortex, which involve motor planning and coordination of movements, as well as nonmotor functions related to cognition and affectivity. In recent years, several studies have suggested that the cerebellum may play a role in regulating visceral functions. Although the specific neural pathways through which these visceral functions are mediated remain unclear, anatomical evidence to support these functions has been supplied by the detection of a feedback circuit that connects bidirectionally the cerebellum and the hypothalamus, the primary integrative center of the autonomic nervous system. This hypothalamocerebellar circuit strongly supports the idea of the cerebellum as a center of the autonomic nervous system.

Keyword: Cerebellum, Hypothalamus, Hypothalamocerebellar circuit, Autonomic nervous system.

1. BACKGROUND

1.1. The Cerebellum as a Center of the Somatic Nervous System

The publication of the influential work *The Cerebellum as a Neuronal Machine* by John C. Eccles, Masao Ito, and Janos Szentagothai in 1967 established the foundations for understanding the anatomy, physiology, pathophysiology, and clinical aspects of the cerebellum. A series of neuroscience studies conducted in subsequent years have widely validated the findings reported in this pivotal study and have led to the modern views according to which the cerebellum is considered to play a crucial role in integrating vestibular and general proprioceptive sensory and regulating the activity of the motor areas of the neocortex. The cerebellum is functionally divided into three regions: the *vestibulocerebellum*, *spinocerebellum*, and *cerebrocerebellum* (also known as *pontocerebellum*) (Table 1).

This functional subdivision is reflected in the anatomical subdivision of the cerebellum (Figure 1). In fact, the vestibulocerebellum substantially cor-

Table 1. Functional subdivision of the cerebellum: main afferents and efferents.

	Afferents	Efferents
Vestibulocerebellum	· Vestibulocerebellar tract · Olivocerebellar tract	· Cerebellovestibular tract · Cerebelloolivay tract
Spinocerebellum	· Posterior spinocerebellar tract · Anterior spinocerebellar tract · Cuneocerebellar tract · Reticulocerebellar tracts · Trigeminocerebellar tracts · Olivocerebellar tract	· Cerebellotectal tract · Cerebellorubral tract · Cerebelloreticular tracts · Cerebelloolivay tract
Cerebrocerebellum	· Pontocerebellar tracts · Reticulocerebellar tracts · Olivocerebellar tract	· Cerebellothalamic tract · Cerebelloreticular tracts · Cerebelloolivay tract

responds to the *flocculonodular lobe*, the spinocerebellum comprises a significant portion of the *anterior lobe* and a portion vermis in the middle lobe, and the cerebrocerebellum includes most of the *middle* (or *posterior*) *lobe* along with portions of the hemispheres in the anterior lobe (Ito, 1984; Berry et al., 1995; Voogd and Glickstein, 1998; Fitzpatrick, 2004; Brodal, 2016; Unverdi et al., 2024). Interestingly, these functional and anatomical subdivisions also have phylogenetic significance. The vestibulocerebellum is the phylogenetically oldest region, known as the *archicerebellum*; the spinocerebellum occupies an intermediate position from a phylogenetic point of view, referred to as the *paleocerebellum*; and the cerebrocerebellum represents the most recent phylogenetically region, termed the *neocerebellum* (Berry et al., 1995).

The vestibulocerebellum and spinocerebellum, especially through the fastigial nucleus and the globose and emboliform nuclei respectively, control various centers of the somatic motor system, including the vestibular nuclei, midbrain tectum, magnocellular red nucleus, and some neuronal groups in the reticular formation (Table 1). Through these centers, the vestibulocerebellum and spinocerebellum regulate the activity of brainstem and spinal somatic motor neurons, which are directly responsible for the contraction of striated muscle fibers. This regulation is crucial for maintaining muscle tone and executing automatic somatic movements. Consequently, both the vestibulocerebellum and spinocerebellum play an essential role in controlling balance, posture, walking, and gaze (Ito, 1984; Berry et al., 1995; Voogd and Glickstein, 1998; Ghez and Thach, 2000; Hook and Mugnaini, 2003; Fitzpatrick, 2004; Voogd and Ruigrok, 2012; Brodal, 2016).

The cerebrocerebellum is part of the cerebrocerebellar circuit, which is a feedback or loop circuit establishing a two-way connection between the neocortex and

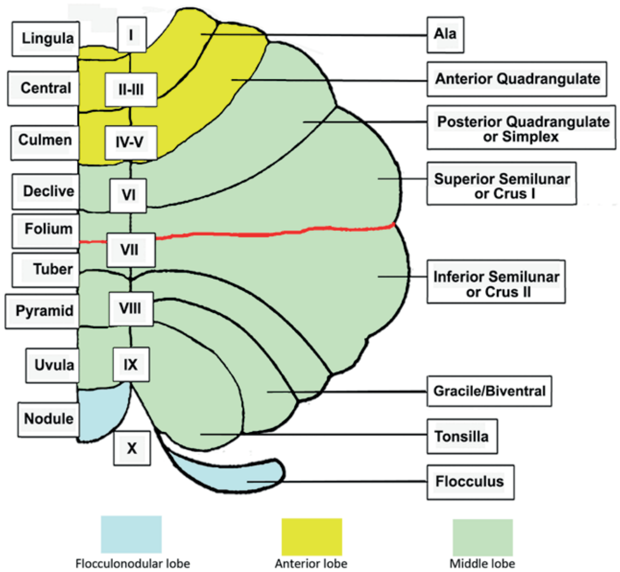

Figure 1. Anatomical subdivisions of the human cerebellum. Diagram showing the lobes and lobules of unfolded cerebellum. The lobules of the vermis and the right hemisphere of the right side are named according to the classic anatomical nomenclature and to the Larsell's nomenclature (Roman numerals from I to X); the horizontal fissure is marked in red. The anatomical of the cerebellum subdivision into three lobes largely corresponds to the functional subdivision of the cerebellum: the vestibulocerebellum relates to the flocculonodular lobe, the spinocerebellum, to the anterior lobe, and the cerebrocerebellum, to the middle lobe.

the cerebrocerebellum. The circuit consists of a descending limb that originates from the neocortex and projects, via the basilar pontine nuclei, onto the cortex of the cerebrocerebellum; and an ascending limb that originates from the cerebrocerebellum, especially from the dentate nucleus, and selectively projects, via the thalamus (ventrolateral nuclear complex), onto the motor areas of the neocortex. This circuit is involved in regulating the activity of neocortical areas responsible for planning complex spatial and temporal sequences of movements as well as executing voluntary movements that require high precision and coordination (Kelly and Strick, 2003; Schmahmann et al., 2004; Fitzpatrick, 2004; Schmahmann and Pandya, 1997a, b; Voogd and Ruigrok, 2012).

Numerous clinical studies have evidenced that cerebellar disorders can be associated with specific clinical symptoms that present a correlation with the anatomical and functional subdivisions of the cerebellum, even if these symptoms may occur in various combinations. In summary, vestibulocerebellar disorders are primarily associated with vestibular symptoms, such as balance defects, dizziness, and nystagmus. Spinocerebellar disorders mainly involve somatic motor symptoms, including disturbances in muscle tone (hypotonia), posture, and gaze issues, as well as difficulties in performing rapid alternating movements (dysdiadochokinesia). Cerebrocerebellar disorders are characterized by motor symptoms resulting from functional deficits in the motor areas of the neocortex. These clinically occur with impairments in executing coordinated and synergistic movements (*motor ataxia*). This provokes disorders of voluntary movements, including walking, praxias, speech articulation, and oculomotion; inability to execute movements that require high precision during their execution, typified by undershooting or overshooting the intended position with the hand, arm, or leg (dysmetria); appearance of tremor during voluntary movements (intention tremor) (Koeppen 2018; Manto et al., 2022; Ataullah et al., 2024).

1.2. The Cerebellum as a Center of the Psychic System

More recent research has shown that the cerebrocerebellum plays a crucial role in regulating functions played by nonmotor areas of the neocortex (Strick et al., 2009; Timman et al., 2010; Grimaldi and Manto, 2012; Benagiano et al., 2018). The anatomical bases for these nonmotor functions of the cerebrocerebellum lie in the organization of the cerebrocerebellar circuit into distinct channels (or subcircuits). Each channel includes a descending limb that originates from a specific nonmotor area of the neocortex and projects onto an anatomically and functionally related region of the cerebrocerebellum; and an ascending limb that starts in the cerebrocerebellum (especially from the dentate nucleus) and projects back onto the same cortical area from which the descending limb originated. Therefore, while the traditional view of the cerebrocerebellar circuit primarily focuses on the motor channel, the new views postulate the existence of nonmotor channels, including the sensory, associative, and limbic channel. These channels establish bidirectional connections between the neocortex of sensory associative and limbic areas and related regions of the cerebrocerebellum (Schmahmann and Pandya, 1991, 1993, 1995, 1997a, b; Clower et al., 2001; Middleton and Strick, 2001; Dum and Strick, 2003; Kelly and Strick, 2003; Ramnani et al., 2006; Akkal et al., 2007; Leergaard and Bjaalie, 2007).

The presence of distinct channels throughout the cerebrocerebellar circuit has been confirmed by studies carried out with diffusion tensor tractography techniques. These studies have revealed that the tracts in the descending limb (neocorticopontine and pontocerebellar tracts) and those in the ascending limb (cerebellothalamic and thalamocortical tracts) are organized into separate, anatomically distinct fascicles. Each of these fascicles connects a specific area of the neocortex with a corresponding region in the cerebrocerebellum (Granziera et al., 2009; Kamali et al., 2010; Kwon et al., 2011; Keser et al., 2015; Palesi et al., 2015).

These observations have provided the anatomical bases for findings from experimental, neuropsychological, and clinical studies, indicating that lesions selectively localized in a specific region of the cerebrocerebellum can disrupt a particular channel and be associated with specific nonmotor disorders. The resulting cerebrocerebellar syndromes can influence cognitive functions, leading to conditions like *cognitive ataxia*, which affects sensory perceptions, learning, memory, language, and ideation (Appollonio et al., 1993; Leiner et al., 1993; Topka et al., 1993; Daum et al., 1993; Akshoomoff and Courchesne, 1994; Silveri and Misciagna, 2000; Gottwald et al., 2004; Schmahmann et al., 2007a, b; Timman and Daum, 2010). Alternatively, the syndromes can impact affective functions, leading to *affective ataxia*, which concerns the adequacy of mood, balance of emotions and feelings, and appropriateness of behavior (Ho et al., 2004; Schmahmann et al., 2007a,b; Tavano et al., 2007; Hoppenbrouwers et al. 2008; Moreno-Lopez et al 2015; Carta et al 2019).

Finally, in cases of neurodevelopmental disorders of the cerebrocerebellum, clinical and behavioral studies have described, in addition to motor ataxia, deficits in learning and concentration classifiable as autism spectrum disorders (Courchesne, 1997; Muratori et al., 2001; Jones et al., 2002; Steinlin, 2008; Bolduc and Limperopolous, 2009; Bolduc et al., 2012; Becker and Stoodley, 2013).

The *Cerebellar Cognitive Affective Syndrome* (CCAS) is a complex clinical syndrome characterized by various symptoms, including disorientation in space and time, difficulty in concentrating (hypoprosexia), challenges in solving logical problems, and deficits in generating, developing, and communicating ideas; patients with CCAS also exhibit inadequate emotional expressions and personality changes. The diverse symptoms associated with CCAS have been linked to dysfunction affecting the different cerebrocerebellar channels (Schmahmann and Sherman, 1998).

Studies using functional magnetic resonance imaging (fMRI) have shown continuous activation of the cerebrocerebellar lobes while performing working memory tasks. This has suggested that the cerebrocerebellum and its connections with various areas of the neocortex play a crucial role in learning and memory processes (Chen and Desmond, 2005; Marvel and Desmond, 2010; von der Gablentz et al., 2015; Peterburs & Desmond, 2016; Brissenden et al., 2021).

1.3. The Cerebellum as a Center of the Autonomic Nervous System

Experimental studies and clinical observations have suggested that the cerebellum plays a role in regulating various visceral functions (Reis and Golanov, 1997; Xu and Frazier, 2000; Colombel et al., 2002; Dietrichs and Haines, 2002; Zhu et al., 2004; Peng et al., 2006; Zhu and Wang, 2008; Cao et al., 2015).

While the idea of the cerebellum acting as a regulatory center for the autonomic nervous system has been proposed for several years, research on the anatomical bases for this function has been surprisingly limited and the available information is scarce and incomplete.

The present review aimed to assess the current understanding of the cerebellum as a regulatory center for visceral functions. Particular attention was paid to exploring whether the somatic, psychic, and autonomic functions of the cerebellum influence one another and what the consequences of these interactions may be.

2. HYPOTHALAMOCEREBELLAR CIRCUIT

The anatomical bases for the role of the cerebellum as a regulatory center of the autonomic nervous system could be supported by the demonstration of the hypothalamocerebellar circuit. This is a feedback circuit that bidirectionally connects the hypothalamus, the main regulatory center of visceral functions, and the cerebellum (Haines et al., 1997; Zhu et al., 2006; Sakakibara, 2018; Rizzi et al., 2020; Urbini et al., 2023).

Similar to the cerebrocerebellar circuit, the hypothalamocerebellar circuit is composed of a descending and an ascending limb. The descending limb includes a direct and an indirect pathway (Figure 2).

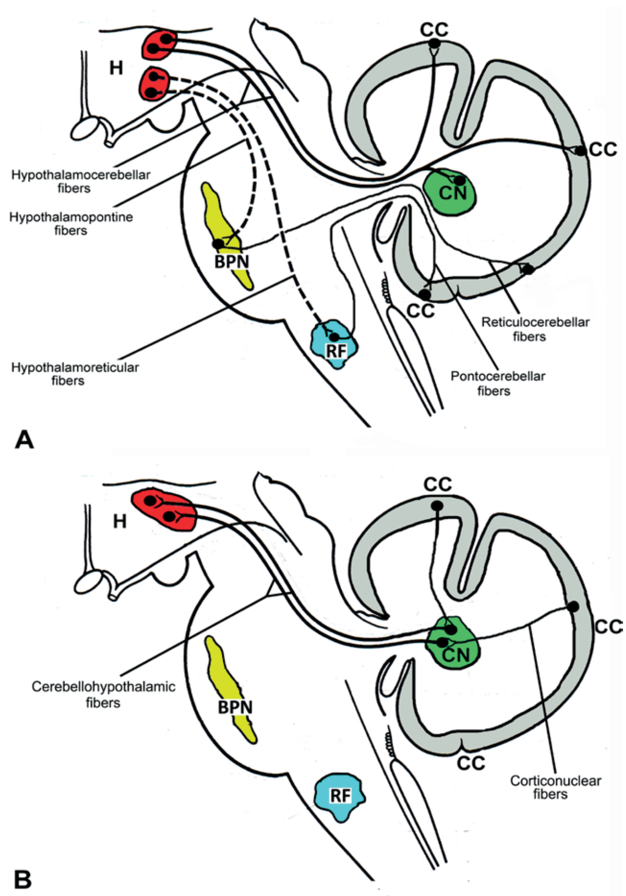

Figure 2. Hypothalamocerebellar circuit: A. descending limb; **B.** ascending limb. The diagram illustrates both the direct and indirect pathways of the circuit. The hypothalamic nuclei putatively at the origin of hypothalamocerebellar, hypothalamoreticular, and hypothalamopontine fibers include the preoptic, ventromedial, dorsomedial, medial mamillary, and tuberomamillary nucleus. **Abbreviations: BPN**: basilar pontine nucleus; **CC**: cerebellar cortex; **CN**: cerebellar nucleus; **H**: hypothalamus; **RF**: reticular formation.

2.1. Direct Hypothalamocerebellar Pathway

The direct hypothalamocerebellar pathway connects the hypothalamus and cerebellum directly and bidirectionally (Benagiano et al., 2018; Rizzi et al., 2020).

Descending limb: hypothalamocerebellar fibers (Figure 2A). Anatomical studies carried out on experimental animals have shown that the descending limb consists of direct hypothalamocerebellar fibers originating from neurons in various hypothalamic nuclei. These nuclei include the preoptic, ventromedial, dorsomedial, medial mamillary, and tuberomamillary nucleus. To a lesser extent, hypothalamocerebellar neurons have also been detected in the suprachiasmatic, posterior, paraventricular, and arcuate nucleus (Dietrichs and Haines, 1984; Haines and Dietrichs, 1984). The hypothalamocerebellar fibers descend to the midbrain tegmentum and prevalently enter the homolateral superior cerebellar peduncle, reaching the central core of the white matter in the cerebellum. Here, they send collaterals to all the cerebellar nuclei and ultimately radiate towards the cortex of *all* the cerebellar lobes (Dietrichs and Haines, 1984; Haines and Dietrichs, 1984; Supple, 1993; Dietrichs et al., 1994; Haas and Panula, 2003; Haas et al., 2008).

Microscopic observations have revealed that the hypothalamocerebellar fibers terminate in all layers of the cerebellar cortex, *multilayered fibers*, possibly synapsing on various types of corticocerebellar neurons (Panula et al., 1993; Li et al., 2014). Consequently, it has been proposed to consider the multilayered fibers as a third type of afferent fibers to the cerebellar cortex, alongside the well-known mossy and climbing fibers (Haines et al., 1997).

Immunocytochemical studies have shown that the terminals of the multilayered fibers are immunoreactive for histamine and use it as a chemical neurotransmitter (Airaksinen et al., 1988; Panula et al., 1993; Li et al., 1999; Rizzi et al., 2019). Accordingly, histamine receptors (H-receptors) have been detected in the cerebellar cortex of various species of mammals, using autoradiographic, immunocytochemical, and *in situ* hybridization techniques. Specifically, H-1 receptors have been found in all the cortical layers, located on the parallel fibers (Rotter and Frostholm, 1986; Traiffort et al., 1994); H-2 receptors have been detected in the Purkinje neuron layer, on the Purkinje neuron bodies and preaxons, and granular layer, on the granule dendrites (Vizuete et al., 1997); H-3 receptors have been detected in the Purkinje neuron layer only (Chazot et al., 2001; Pillot et al., 2002), and have been visualized in the human cerebellum by positron emission tomography (PET) (Ashworth et al., 2010). In vitro studies have shown that histaminergic terminals have excitatory effects on H-1 and H-2 receptors expressed by granules (Li et al., 1999), and on H-2 receptors expressed by Purkinje neurons (Tian et al., 2000).

Histamine-containing neurons have been demonstrated in some hypothalamic nuclei, primarily in the tuberomamillary nucleus, and to a lesser extent, the ventromedial, dorsomedial, and paraventricular nucleus (Brown et al., 2001; Haas & Panula, 2003; Haas et al., 2008). It is noteworthy that these nuclei also contain the neurons at the origin of the hypothalamocerebellar fibers.

Ascending limb: cerebellohypothalamic fibers (Figure 2B). The excitatory signals sent by the histaminergic terminals of the multilayered fibers activate the Purkinje neurons either directly or indirectly. These neurons serve as the source of output from the cerebellar cortex, sending corticonuclear fibers to the neurons located in all the cerebellar nuclei (Ito, 1984; Berry et al., 1995; Voogd and Glickstein, 1998). It is well known that the outputs from Purkinje neurons to the nuclear neurons are inhibitory and use GABA as a neurotransmitter (Benagiano et al., 2000, 2001).

Anatomical studies using anterograde and retrograde tract-tracing techniques, along with physiological studies based on electrophysiology techniques, have identified the *cerebellohypothalamic fibers*. These fibers originate from neurons located in all the cerebellar nuclei, primarily the fastigium and interpositus nucleus. They travel through the superior cerebellar peduncle, midbrain tegmentum, and into the hypothalamus (Dietrichs and Haines, 1984; Haines and Dietrichs, 1984; Wang et al., 1997; Cavdar et al., 2001a, b). Most of the cerebellohypothalamic fibers cross at the decussation of the midbrain tegmentum, and reach the contralateral hypothalamus, differently from the hypothalamocerebellar fibers, which establish homolateral connections (Lemaire et al., 2011). These fibers project onto hypothalamic nuclei that correspond largely to those at the origin of the descending limb, including the ventromedial, dorsomedial, and tuberomamillary nucleus (Wang et al., 1997; Cavdar et al., 2001a, b). The effects of the cerebellohypothalamic fibers on hypothalamic neurons can be of excitatory type, mediated by glutamate (Lu et al., 2012; Cao et al., 2015), or, more rarely, of inhibitory type, mediated by GABA (Wang et al., 2011; Cao et al., 2013; Lu et al., 2015).

2.2. Indirect Hypothalamocerebellar Pathways

In the indirect pathways, the connections between the hypothalamus and cerebellum are established through the involvement of brainstem centers. Briefly, fibers originating in the same hypothalamic nuclei that give rise to the direct hypothalamocerebellar pathway, before reaching the cerebellum, interrupt in brainstem nuclei, such as nuclei of the reticular formation and basilar pontine nuclei.

The indirect pathways include the *hypothalamoreticulocerebellar* and *hypothalamopontocerebellar pathways*. Each of these pathways consists of a proximal segment, either the hypothalamoreticular fibers or the hypothalamopontine fibers, and a distal segment, either the reticulocerebellar fibers or the pontocerebellar fibers. The *hypothalamoreticular fibers* primarily project onto neurons located in the precerebellar lateral reticular nucleus, which is located in the lateral medullary reticular formation (Dietrichs et al., 1985; Mihailoff et al., 1989; Allen and Hopkins, 1990); the *hypothalamopontine fibers* target neurons that are sparse in the rostral medial and dorsal medial basilar pontine nuclei (Aas, 1989; Liu and Mihailoff, 1999). Actually, detailed information on the anatomy, neurochemistry, and physiology of these pathways is lacking in the literature.

3. DISCUSSION

The existence of a hypothalamocerebellar circuit, which connects bidirectionally the hypothalamus and the cerebellum, is now well established (Dietrichs and Haines, 1984; Haines and Dietrichs, 1984; Supple, 1993; Dietrichs et al., 1994). This is a feedback circuit organized similarly to the more widely recognized cerebrocerebellar circuit. It consists of a descending limb that originates from various hypothalamic nuclei, specifically, the ventromedial, dorsomedial, and tuberomamillary nucleus, and terminates in the cerebellar cortex across all cerebellar lobes; and an ascending limb that starts in the cerebellar cortex and projects back onto the same hypothalamic nuclei from which the descending limb originates. It is important to note that the hypothalamocerebellar fibers are distributed throughout all cerebellar lobes. They terminate as multilayered fibers in all cortical layers and express histamine as a chemical neurotransmitter (Li et al., 1999; Haas and Panula, 2003; Haas et al., 2008; Rizzi et al., 2019). All these are characteristics that differentiate the hypothalamocerebellar fibers, which form the hypothalamocerebellar circuit, from the pontocerebellar ones, which form the cerebrocerebellar circuit.

The hypothalamus acts as the primary regulatory center for visceral functions and is an important subcortical component of the limbic system (Onat and Cavdar, 2003; Saper, 2012). It is likely that the signals sent to the cerebellum via the hypothalamocerebellar fibers, which form the descending limb of the hypothalamocerebellar circuit, contain information related to the visceral and limbic systems. These connections suggest that the cerebellum, through the cerebellohypothalamic fibers, ascending limb of the hypothalamocerebellar circuit, plays a regulatory role of hypothalamic nuclei involved in visceral and limbic functions.

The role of the hypothalamic-cerebellar circuit would be comparable to that of the cerebrocerebellar circuit: the cerebellum could influence the activity of the hypothalamic nuclei just as the cerebrocerebellum controls the activity of motor and nonmotor areas of the neocortex. Interestingly, the entire cerebellum would be involved in regulating the hypothalamus, while only the cerebrocerebellum would be involved in regulating the neocortex.

The existence of the hypothalamocerebellar circuit provides anatomical evidence supporting a series of observations that indicate the role of the cerebellum in regulating visceral functions and its involvement in the pathogenesis of visceral disorders. These findings align with experimental studies showing that electrical stimulation or lesions of the cerebellum can trigger visceral changes affecting gastrointestinal, cardiovascular, respiratory, immune, and other visceral functions (Reis and Golanov, 1997; Xu and Frazier, 2000; Colombel et al., 2002; Dietrichs and Haines, 2002; Zhu et al., 2004; Peng et al., 2006; Zhu and Wang, 2007; Cao et al., 2015).

Additionally, the hypothalamus is a center of the limbic system, which is extensively connected to other limbic centers, including the limbic lobe of the neocortex, hippocampus, amygdala, and ventral striatum. This suggests that the hypothalamocerebellar circuit may play a role in regulating psychic functions related to mood, emotions, feelings, and instinctive behaviors (Ho et al., 2004; Schutter and van Honk, 2005; Schmahmann et al., 2007; Tavano et al., 2007; Hoppenbrouwers et al., 2008; Moreno-Lopez et al., 2015; Carta et al., 2019). These functions, influenced by the hypothalamocerebellar circuit, would complement those exerted through the limbic channel of the cerebrocerebellar circuit.

The hypothalamocerebellar fibers connect the hypothalamus with all lobes of the cerebellum. This means they inevitably terminate in regions of the cerebellum that also receive other types of inputs, i.e., vestibular afferents (vestibulocerebellum), general proprioceptive afferents (spinocerebellum), and cerebrocerebellar afferents (cerebrocerebellum). The overlap of these different types of afferents in the same regions of the cerebellar cortex enhances the interaction among the somatic, psychic, and visceral functions regulated by the cerebellum. The integration of the vestibular sensory, played by the vestibulocerebellum, and that of the general proprioceptive sensory, played by the spinocerebellum, would be influenced by visceral sensory information from the hypothalamus, which reach the vestibulocerebellum and the spinocerebellum. In turn, both the vestibulocerebellum and spinocerebellum send back to the hypothalamus signals that would influence its visceral motor responses. On the other hand, the regulatory function of the neocortex, played by the cerebrocerebellum through the cerebrocerebellar circuit, would be influenced by visceral sensory information, leading to complex interactions between the somatic, psychic, and autonomic system.

Finally, the basilar pontine nuclei and some neuronal groups within the reticular formation, which are intercalated in the descending limb of the indirect hypothalamocerebellar pathway and the cerebrocerebellar circuits, could serve as further points of contact between the two main regulatory circuits within the central nervous system.

4. CONCLUSION

The cerebellum can be regarded as a central component of the somatic, psychic, and autonomic systems. It plays a significant role in regulating various areas of the neocortex, through the cerebrocerebellar circuit, as well as different hypothalamic nuclei, through the hypothalamocerebellar circuit.

These new perspectives on the cerebellum functions may help explain the development of visceral disorders, mood disorders, and behavioral disturbances, commonly observed in patients with cerebellar diseases, alongside the symptoms commonly associated with these conditions.

Unfortunately, these insights have not yet gained widespread recognition in clinical settings, where the cerebellum is often viewed solely in terms of its motor functions. It is crucial that further experimental, behavioral, and clinical studies support these new perspectives so that they can be effectively integrated into clinical practice.

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IJAE
ITALIAN JOURNAL OF
ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Alberti, P. (2024). 3D Digital tools in neuro-anatomy teaching: from *peer-to-peer* tutoring to clinically oriented approaches. *Italian Journal of Anatomy and Embryology* 128(2): 125-134. https://doi.org/10.36253/ijae-15763

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

3D Digital tools in neuro-anatomy teaching: from *peer-to-peer* tutoring to clinically oriented approaches

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Abstract. Neuroanatomy knowledge is pivotal due to the constantly increased use of neuroimaging in everyday clinical practice, even in the emergency setting as it is for patients potentially affected by stroke. To empower our students, we are relying on innovative 3D digital tools to teach human anatomy, exploiting several approaches among which *Anatomage Table*™, also fostering a *peer-to-peer* tutor program. We present here the results of a pilot phase of these initiatives. First year med students from the Italian and International Course used *Anatomage Table*™ under the supervision of fourth-, fifth-, or sixth-year med students, as *peer-to-peer* tutors. Participants were divided into groups (10-12 people each). Three sessions lasting 2 hours were planned with a predefined topic: thorax (topic 1), abdomen/pelvis (topic 2), neuroanatomy (topic 3). A questionnaire was filled at the end of each session rating satisfaction/impact. One-hundred-thirty students (105 from the Italian course and 25 from the international course) participated. More than 90% of students rated the neuroanatomy session as highly satisfactory and more than 95% as highly useful, and 100% of them would suggest others participate. Based on these results, a further implementation is planned for the next year, also exploiting DICOM data to present the students with real clinical cases bearing a highly didactive anatomical content. Specifically, in the international course, based on a vertical track approach, we are also strongly relying on flipped-classroom approach. Digital tools to teach neuroanatomy are also being implemented for clinically oriented teaching for residents/consultants.

Keywords: human anatomy, virtual dissection, innovative teaching methods, Anatomage™ table, peer-to-peer tutoring.

INTRODUCTION

Neuroanatomy is pivotal in the daily management of patients affected by disorders involving the central and/or the peripheral nervous system: a detailed and robust understanding of neuroanatomical aspects is an essential guide to correctly perform and interpret the neurological examination and then appropriately run diagnostic exams, since most of them have a high neuroanatomical correlation, such as neuroimaging [1-5] (Computer Tomography [CT] scan, magnetic resonance imaging [MRI], nuclear medicine techniques, and ultrasounds [US]) and neurophysiological techniques [6].

Moreover, neuroanatomy has gained an even more relevant role in everyday clinical practice and not only for specialties directly related to neuro-psychiatric disorders (e.g., neurology, neurosurgery, psychiatry, childhood psychiatry/neurology, neuroradiology, …), given advancements in acute stroke care [7-10]: thrombolytic agents as well as mechanical recanalisation of large blood vessel occlusions have been a game changer in acute ischemic stroke management. This led to the implementation of an emergency system that involves different health-care professionals, making not-negotiable a robust neuroanatomical knowledge (https://www.agenas.gov.it/comunicazione/primo-piano/2233-reti-ictus-presentazione-relazione-conclusiva-del-board-agenas).

Another example of a wider use of neuroanatomy by health care professionals different from neurologists/neurosurgeons is the implementation of US to study the peripheral nervous system: on top of the most obvious diagnostic application for diagnosis of neuropathy [3,4], US can be used, in fact, to guide procedures such as local anesthesia and pain management targeting a specific branch of the peripheral nervous system [11-14].

Therefore, this highlights once again the relevance of neuroanatomy for a broader audience of health care providers. To build a robust knowledge, not faltering over the years, exposure to clinically oriented neuroanatomy is essential as soon as the first years of training, possibly relying on advanced, innovative and digital approaches, especially in the lack of easy access to a fully-equipped dissection facility; in Italy, in fact, despite the approval in 2020 of a law on body donation [15], it is still difficult in most Centers to widely implement real dissections in all human anatomy teachings.

In our University, *Anatomage Table*^{TM} has been implemented in the last few years: *the Anatomage Table*^{TM} Software (*Anatomage Inc*., Santa Clara, California, US) allows 3-D reconstructions of 5 different segmented cadavers, enabling hands-on virtual dissections. Moreover, it contains (in its own case library) and allows the upload of Digital Imaging and Communication in Medicine (DICOM) data to be then reconstructed with photorealistic filters.

We present here a pilot experience relying on digital instruments, aiming at enabling students to be an active element in the process of learning via a *peer-to-peer* tutoring activity.

METHODS

Population

First year students in Medicine and Surgery were offered the opportunity to participate in this initiative as an extracurricular, not mandatory, activity. Students were enrolled from the two different master's degrees in medicine and surgery available at our university: the standard course, named from now as the *Italian* course, and the *International* course (all teaching activities are carried out in English language). In the former, students receive human anatomy teachings as a single course during the first year, equivalent to 20 European Credit Transfer and Accumulation System (ECTS), with a horizontal track approach. Whereas, in the latter students are given a more basic course during the first year, named *Fundamentals of Human Morphology* (8 ECTS), and receive further human anatomy teachings in the subsequent years with a vertical track approach; this means, as an example, that in the Neuroscience vertical track, students will receive 4 more ECTS only on neuroanatomy.

Peer-to-peer tutors' selection and training

Both cohorts were given the opportunity to take part in *peer-to-peer* activities during the second semester of the first year, after the topics were already covered in frontal lessons. They were allowed to actively cooperate with a fourth-, fifth-, or sixth-year med student, among a pool of nine tutors who were selected via a public call among those who already passed the human anatomy full exam. Tutors participated in 10 hours of training and were then available for 2-hours sessions (10 each). *Peer-to-peer tutor* training was performed both on-line and on-site. Tutors independently completed a training course available on *Anatomage Share – Training Portal*, provided by *Anatomage Inc*. free of charge (https://www.anatomageshare.com/training-portal) and a 1-hour zoom call was scheduled to revise together the main functionalities. The on-site training was then performed directly on *Anatomage Table*^{TM}: the first 2 hours were intended to make tutors confident in using all the functions of the tools and other sessions were then organised with a professor to harmonise all tutors' actions for the three different topics to be revised with students (see the following section). The instructor devised a well-defined workflow for all the topics and prepared presets/selected specific visualization to maximize the impact of all activities and ensure all students received the same information regardless of the tutor they worked with. All tutors were provided with detailed instructions on the workflow to be followed.

Activities organisation

Students from the Italian cohort were named as *Cohort-ITA* and ones from the international course as *Cohort-ENG*; activities were held separately for the two cohorts, since for *Cohort-ENG* all activities were carried out in English language, while for Cohort-ITA in Italian language. Participants from both cohorts were divided into groups (no more than 10-12 students each). Three sessions lasting two hours were planned. The first session was mostly dedicated to revising the thorax region and the hearth (*Topic 1*), in the second session the abdomen/pelvis regions were revised (*Topic 2*), and in the third session neuroanatomy was revised (*Topic 3*).

Data collection and analysis

Data was collected anonymously via a Google module made accessible via a QR code. Participants were asked to fill in a questionnaire before starting the first session and at the end of each session, using their cell phones. Questions were meant to explore the impact and satisfaction of the initiatives carried out. The same grading system (on a 0-10 scale) used by our university to rate students' opinion on didactic activities was followed (https://opinionistudenti.unimib.it/valdid/): low (0-4), intermediate (5-7), and high score (8-10).

A descriptive statistic of all quantitative data was generated and differences among different topics/cohorts were tested with parametric test or non-parametric test based on the distribution of the data (normally or not normally distributed respectively), setting a 2-sided test (significance at p-value 0.05).

RESULTS

Overall, 130 students took part into the initiative: 105 (62%) students of Italian course and 25 (52%) of the international course. In Table 1 satisfaction and usefulness descriptive statistics and Mann-Whitney U-test results are presented.

Preliminary survey before starting the first session

Before starting Topic 1 activities, students were asked about the frequency of their use of the anatomy room for independent study: we obtained 47 replies from Cohort-ITA and 21 replies from Cohort-ENG (results are summarised in Figure 1). For what regards Cohort-ITA: 55.4% never used the room and did not plan to use it later in the semester, 19.1% never used the room but planned to use it later in the semester, 14.9% already used rarely the anatomy room for independent study, 10.6% did not know they could use the room for independent study. For what regards Cohort-ENG: 57.1% never used the room but planned to use it later in the semester and 42.9% did not know they could use the room for independent study. The perceived usefulness of the current opportunity offered by the anatomy room (i.e., without the possibility of exploiting *Anatomage™ Table*) was also tested (see Figure 2): the median

Table 1. Descriptive statistics and Mann-Whitney U-test results for the survey questions assessing satisfaction and perceived usefulness.

Item	Cohort	Time point	Median score	Q1	Q2	Mann-Whitney U-test (p-value)
Perceived usefulness of the anatomy room with no use of the Anatomage™ table	Cohort-ITA	T_0	7	5	8	0.9493
Perceived usefulness of the anatomy room Anatomage™ table	Cohort-ENG	T_0	7	4.5	8	
Satisfaction regarding the presented topic	Cohort-ITA	T_1	9	8.5	10	0.3576
Satisfaction regarding the presented topic	Cohort-ENG	T_1	9	8	10	
Perceived usefulness of the presented topic	Cohort-ITA	T_1	9	8	10	0.6378
Perceived usefulness of the presented topic	Cohort-ENG	T_1	9	8	10	
Satisfaction regarding the presented topic	Cohort-ITA	T_2	10	9	10	**0.0481**
Satisfaction regarding the presented topic	Cohort-ENG	T_2	9	8	10	
Perceived usefulness of the presented topic	Cohort-ITA	T_2	10	9	10	**0.0276**
Perceived usefulness of the presented topic	Cohort-ENG	T_2	9	8	10	
Satisfaction regarding the presented topic	Cohort-ITA	T_3	9	8	10	0.5795
Satisfaction regarding the presented topic	Cohort-ENG	T_3	9	8	10	
Perceived usefulness of the presented topic	Cohort-ITA	T_3	9	8	10	0.5683
Perceived usefulness of the presented topic	Cohort-ENG	T_3	9	8.5	10	

T_0: before starting the whole initiative; T_1: after completing Topic $_1$; T_2: after completing Topic 2; T_3: after completing Topic 3.

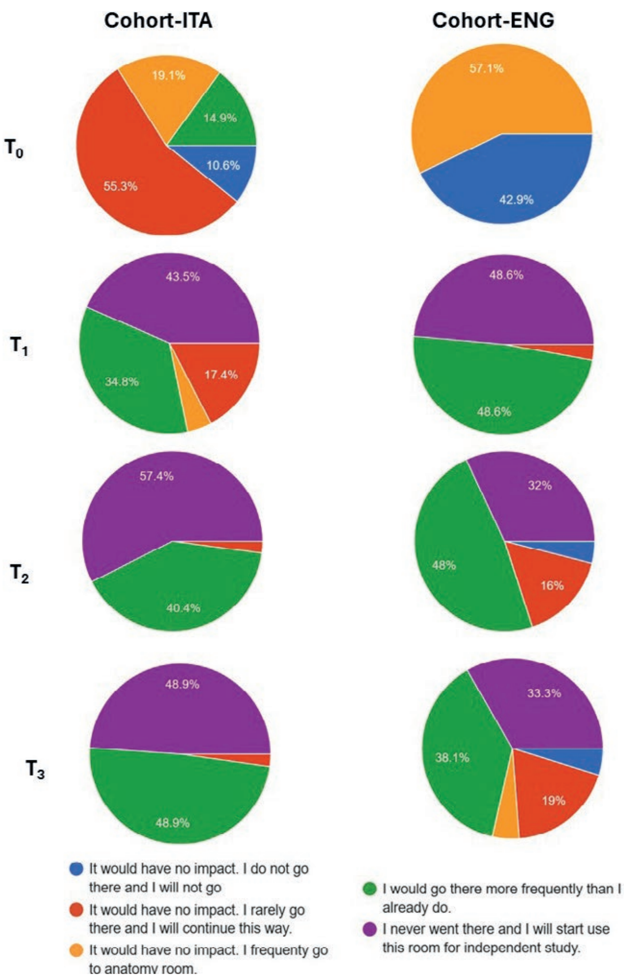

Figure 1. Willingness of the students to use the anatomy room for independent study. T_0: before starting the initiative; T_1: after completing Topic $_1$; T_2: after completing Topic 2; T_3: after completing Topic 3.

score assigned by Cohort-ITA was 7 (Q1:5; Q3: 8) and by Cohort-ENG was also 7 (Q1: 4.5; Q3: 8); more in details: 8% of Cohort-ITA assigned a low score (i.e., score values 0-3), 58% an intermediate score (i.e., score values 4-7), and 34% a high score (i.e., 8-10), while for Cohort-ENG the proportion of different score classes were respectively 19%, 38%, 43%. No statistically significant differences were observed between the 2 cohorts.

Survey results after Topic 1 activities

After completing Topic 1 activities, level of satisfaction and usefulness were evaluated: 105 responses were collected from Cohort-ITA and 23 from Cohort-ENG. For what regards satisfaction (see Figure 3), Cohort-ITA assigned a median score of 9 (Q1: 8.5; Q3: 10), with 94% of replies being set in the highest score class and 6% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8; Q3: 10), with 96% of replies being set in the highest score class and 4% in the intermediate score class. No statistically significant differences were observed between the 2 cohorts.

For what regards perceived usefulness, Cohort-ITA assigned a median score of 9 (Q1: 8; Q3: 10), with 86% of replies being set in the highest score class and 12% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8; Q3: 10), with 96% of replies being set in the highest score class and 4% in the intermediate score class. No statistically significant differences were observed between the 2 cohorts.

Participants were then asked if they would implement more independent study in the anatomy room if the *Anatomage*^{TM} *table* was made available. Cohort-ITA replies can be summarised as follows: 48.6% stated that would increase the frequency of their access shifting from no access at all to going to the anatomy room for

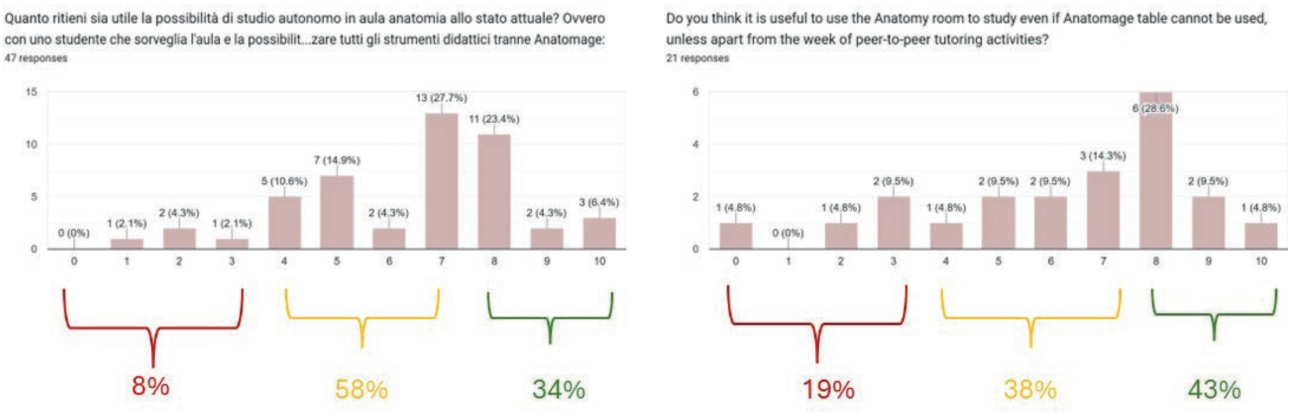

Figure 2. Perceived usefulness of the access in the anatomy room without the opportunity to use the *Anatomage Table*^{TM}.

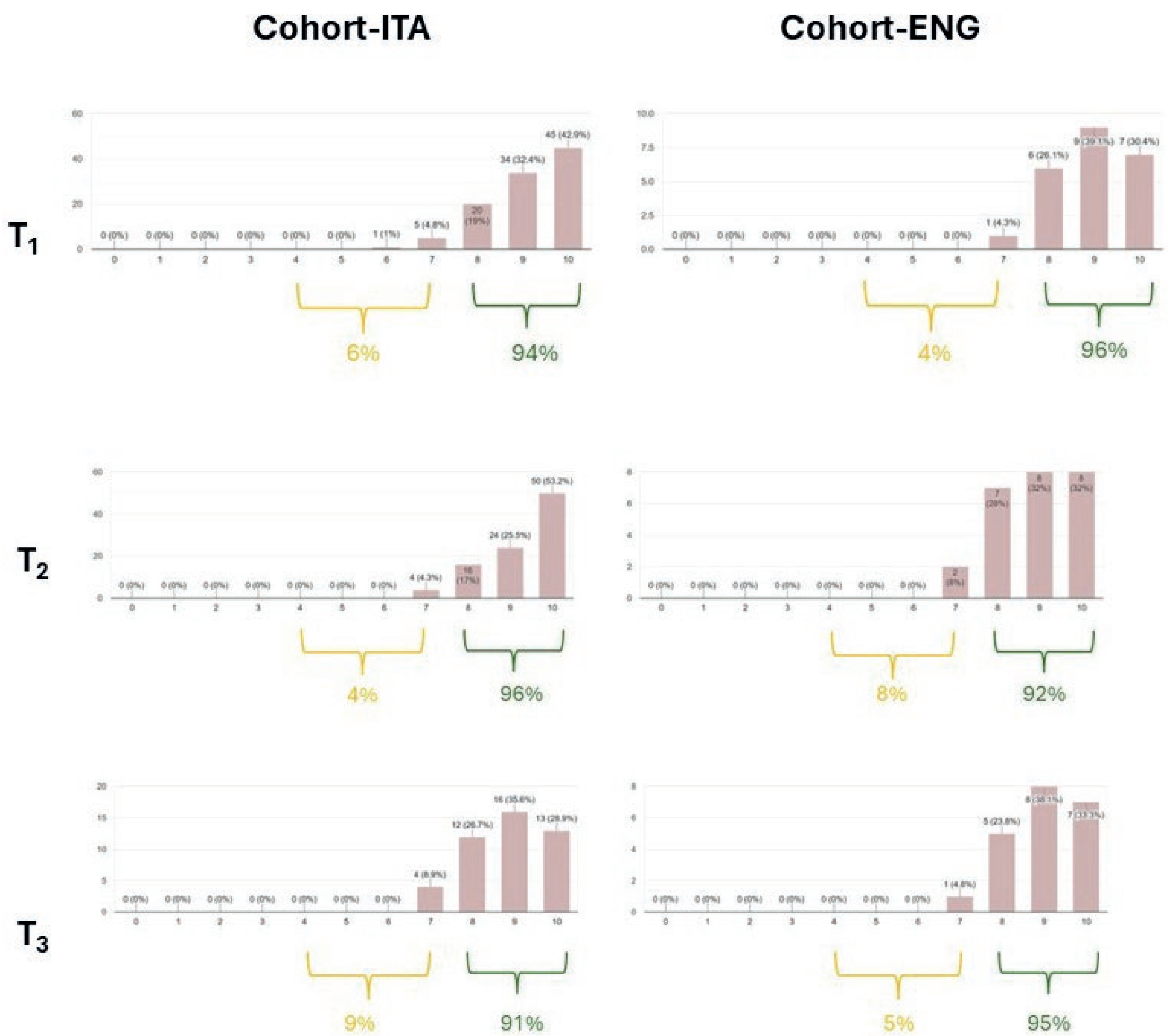

Figure 3. Satisfaction of the students regarding the activities. T_0: before starting the initiative; T_1: after completing Topic $_1$; T_2: after completing Topic 2; T_3: after completing Topic 3.

independent study if this tool were to be made available, 48.6% stated that would increase the frequency of their access shifting from rare access to a more frequent access, 2.8% stated that they rarely used the room and the frequency of access would be the same. Cohort-ENG replies can be summarised as follows (see Figure 1): 43.5% stated that would increase the frequency of their access shifting from no access at all to going to the anatomy room for independent study if this tool were to be made available, 34.8% stated that would increase the frequency of their access shifting from rare access to a more frequent access, 17.4% stated that no impact on the current frequency (rare) of access would be in place, and 4.3% % stated that no impact on the current frequency (quite frequent) of access would be in place.

Participants were also asked to state if they would suggest others participate after completing this activity: 100% replied they would in both cohorts (see Figure 5).

Survey results after Topic 2 activities

After completing Topic 2 activities, level of satisfaction and usefulness were evaluated: 94 responses were collected from Cohort-ITA and 25 from Cohort-ENG (see Figure 3). For what regards satisfaction, Cohort-

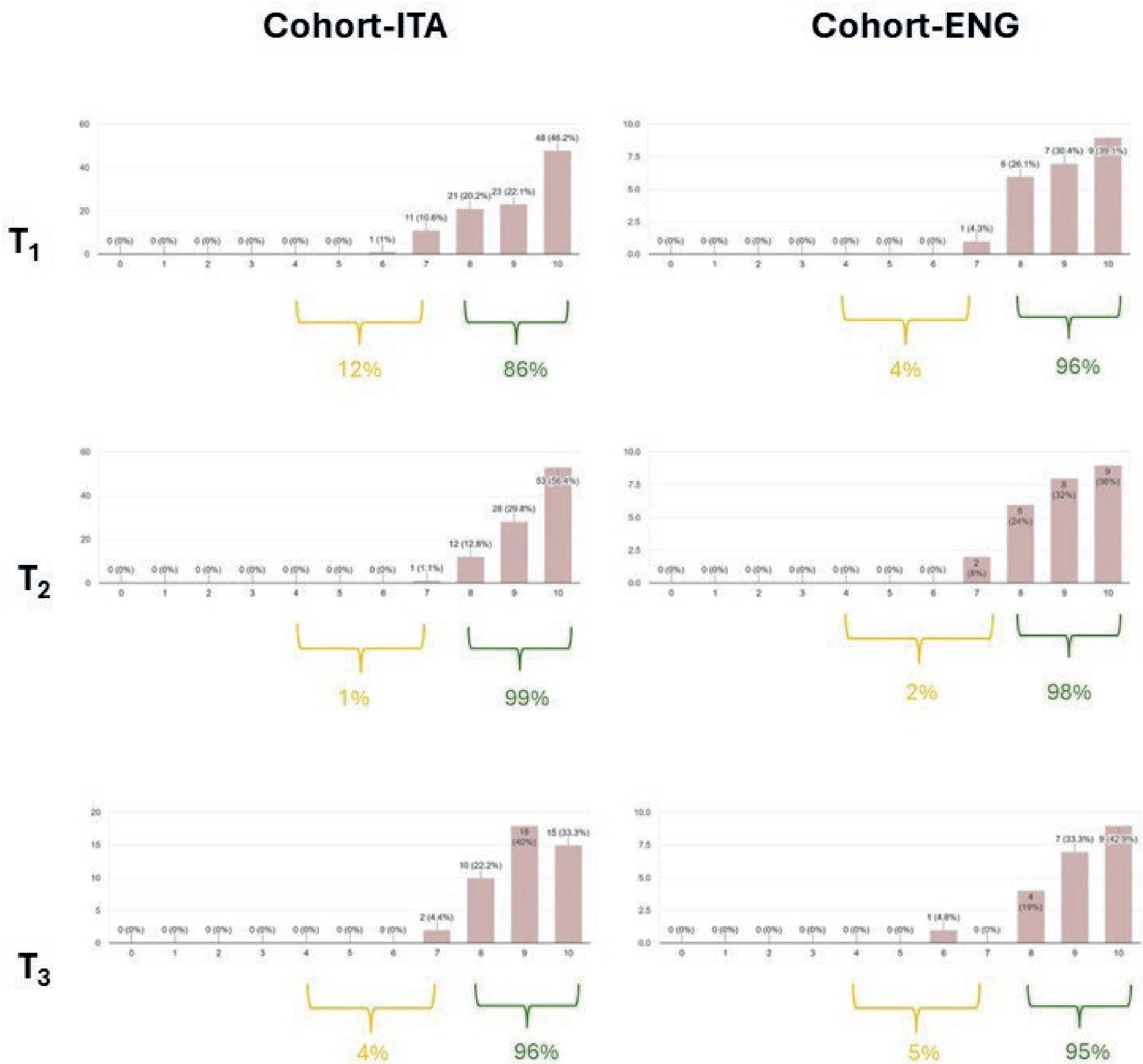

Figure 4. The usefulness of the activities perceived by the students. T_0: before starting the initiative; T_1: after completing Topic $_1$; T_2: after completing Topic 2; T_3: after completing Topic 3.

ITA assigned a median score of 10 (Q1: 9; Q3: 10), with 96% of replies being set in the highest score class and 4% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8; Q3: 10), with 92% of replies being set in the highest score class and 8% in the intermediate score class. A slight statistically significant difference we observed between the 2 cohorts (p-value: 0.0481, Mann-Whitney U-test).

For what regards perceived usefulness (see Figure 4), Cohort-ITA assigned a median score of 10 (Q1: 9; Q3: 10), with 99% of replies being set in the highest score class and 1% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8; Q3: 10), with 98% of replies being set in the highest score class and 2% in the intermediate score class. A Statistically significant difference was observed between the 2 cohorts (p-value: 0.0276, Mann-Whitney U-test).

Participants were then asked if they would implement more independent study in the anatomy room if the *Anatomage^{TM} table* was made available (see Figure 1). Cohort-ITA replies can be summarised as follows: 57.4% stated that would increase the frequency of their access shifting from rare access to a more frequent access, 40.4% stated that would increase the frequency of their access shifting from no access at all to going to the anatomy room for independent study if this tool were to be made available, 2.2% stated that they rarely used the room and the frequency of access would be the same. Cohort-ENG replies can be summarised as follows: 48% stated that would increase the frequency of their access, 32% stated that would increase the frequency of their access shifting from no access at all to a more frequent access, 16% stated that no impact on the current frequency (rare) of access would be in place, and 4% stated that no impact on the current frequency (no access at all) of access would be in place.

Participants were also asked to state if they would suggest others participate after completing this activity: 100% replied they would in both cohorts (see Figure 5).

Survey results after Topic 3 activities

After completing Topic 3 activities, level of satisfaction and usefulness were evaluated: 45 responses were collected from Cohort-ITA and 21 from Cohort-ENG. For what regards satisfaction (see Figure 3), Cohort-ITA assigned a median score of 9 (Q1: 8; Q3: 10), with 91% of replies being set in the highest score class and 9% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8; Q3: 10), with 95% of replies being set in the highest score class and 5% in the intermediate score class. No statistically significant differences were observed between the 2 cohorts.

For what regards perceived usefulness (see Figure 4), Cohort-ITA assigned a median score of 9 (Q1: 8; Q3: 10), with 96% of replies being set in the highest score class and 4% in the intermediate score class; whereas, Cohort-ENG assigned a median score of 9 (Q1: 8.5; Q3: 10), with 95% of replies being set in the highest score class and 5% in the intermediate score class. No statistically significant differences were observed between the 2 cohorts.

Participants were then asked if they would implement more independent study in the anatomy room if the *Anatomage^{TM} table* was made available (see Figure 1). Cohort-ITA replies can be summarised as follows: 48.9% stated that would increase the frequency of their access shifting from no access at all to going to the anatomy room for independent study if this tool were to be made available, 48.9% stated that would increase the frequency of their access shifting from rare access to a more frequent access, 2.2% stated that they rarely used the room and the frequency of access would be the same. Cohort-ENG replies can be summarised as follows: 38.1% stated that would increase the frequency of their access shifting from rare to a more frequent access, 33.3% stated that would increase the frequency of their access shifting from no access at all to a more frequent access, 19% stated that no impact on the current frequency (rare) of access would be in place, 4.8% stated that they would not start to use the room for independent study, and 4.8% stated that no impact on the current frequency (quite frequent) of access would be in place.

Participants were also asked to state if they would suggest others participate after completing this activity: 100% replied they would in both cohorts (see Figure 5).

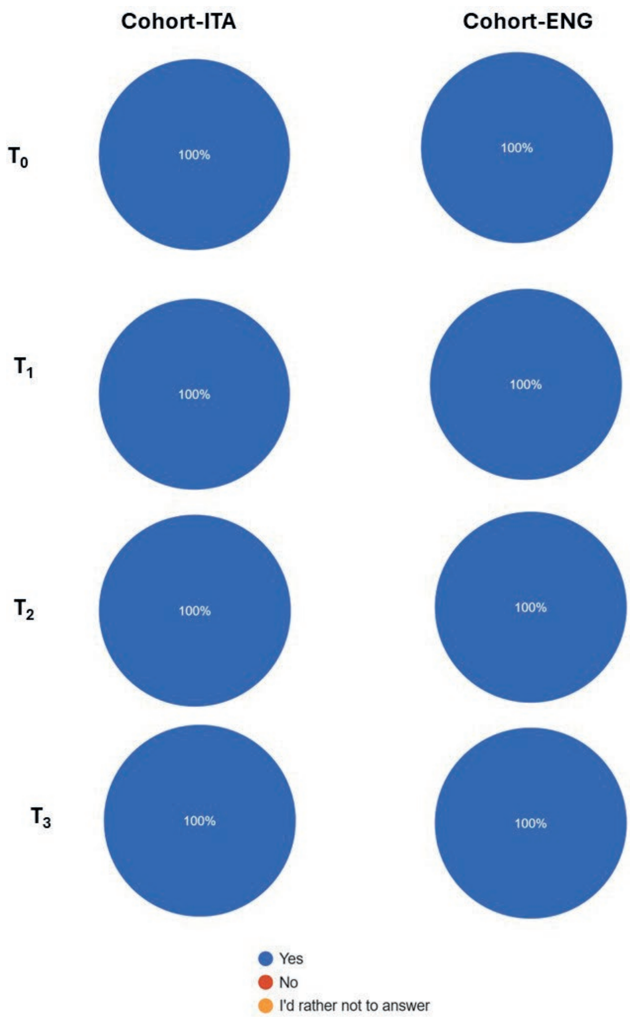

Figure 5. Replies to the question: "Would you suggest others to participate in an initiative such as this?". T_0: before starting the initiative; T_1: after completing Topic $_1$; T_2: after completing Topic 2; T_3: after completing Topic 3.

DISCUSSION

The pilot experience presented here was broader in topics than neuroanatomy, but it is a fair example of how innovative approaches could be eventually implemented in neuroanatomy teachings stemming from positive results as the ones shown above. Both cohorts, in fact, gave back a feedback rating both satisfaction and perceived usefulness as high and no item was rated in the low score category. Comparing all topics intra- and inter-cohorts emerged that *Topic 2* (abdomen/pelvis) received a slightly higher appreciation in Cohort-ITA but in general scores were more than satisfactory as already stated. Furthermore, all participants agreed on suggesting others take part in similar initiatives with no exceptions. Quite striking is also the relevant increase in access to the anatomy room if the *Anatomage^{TM} table* were to be made available for independent study, shifting for a low rate to a high rate of exploitation of this room and its tools during exam preparation. This is even more impressive for the Cohort-ENG since teachings for this course are usually located in Bergamo (Polo di Formazione Universitaria *Papa Giovanni XXIII*), while the anatomy room is in Monza (Asclepio/u8 building); therefore, students from this cohort accepted the logistic nuisance of travelling to exploit what offered in the anatomy room. The relevance of the initiative can also be perceived for both cohorts evaluating the increasing percentage of students willing to exploit the anatomy room in case *Anatomage^{TM} table* was made available for independent study.

Of course, the data presented here is just part of an exploratory study and some limitations should be recognised. First, the number of questionnaires available for evaluation each time was different for each cohort and the numerosity of the 2 cohorts is quite different, making comparison potentially less robust. Second, we measured cross-sectionally students' perceptions of the usefulness and satisfaction for the initiative, but we did not make any correlations with the final mark received at the exam and the participation or not to the initiative. Last, but not least, we did not make any comparison with other possible interactive teaching methods (e.g., paper copy exercise, use of 3D plastic models/dummies, …).

However, data presented here are in line with previous studies showing how virtual dissections and tools can enhance anatomy learning either in case of the availability of real dissections or in case they are not a feasible option [16-29]. In particular, Singer et al. [30] showed how these virtual approaches could be pivotal in enhancing engagement of students while learning anatomy, conducting a study to evaluate students' perception of virtual anatomy platforms.

Therefore, it could be suggested that this kind of approach can empower students and engage them more and provide a direct link to a clinically oriented approach in neuroanatomy exploitation in their subsequent career as health care professionals. In the past few years, in fact, *Anatomage^{TM} Table* was used to tailor approaches in different pathologies with a highly relevant anatomical impact related to the head and neck region [31-34]. More specifically related to neuroanatomy, Sadiq et al. [35] showed how case-based learning exploiting virtual 3D tools enabled to ameliorate neuroanatomy and neuroradiology teachings. Nyangoh Timoh et al. showed the potential application of digital tools to enhance the understanding and then guide clinical procedures related to the excision of endometriosis nodules of the sciatic nerve [36]. Ahmed et al. showed the potential implications of digital tools to ameliorate procedures such as anterior petrosectomy of the skull [37]. Robin et al. implemented the virtual dissection to better understand the anatomical relations between uterine veins, ureter and hypogastric nerve for uterine transplantation providing robust knowledge to proceed to such a delicate procedure [38]. Strantzias et al. [39] proposed the use of the virtual reconstruction via the Table to manage the anatomical variations of the marginal mandibular nerve during surgery. La Torre et al. [40] tested this tool to provide a 3D anatomical visualization to a neurosurgical procedure, the retrosigmod approach, to prevent injuries to the great occipital, lesser occipital, and greater auricular nerves.

In line with all these previous exploitations of the *Anatomage^{TM} Table*, we are implementing its use in all courses relying on advanced neuroanatomy knowledge. The international course in our university, in fact, proposes a vertical track named *Neuroscience* (4th-5th year). In this case, students have already received basic neuroanatomy teachings during the first year, therefore we can implement the use of the table even further, relying mostly on virtual dissections during the teachings rather than the classical slide deck with plain 2D images; an opportunity easy to provide since the number of students is quite limited (maximum per year: 48). Therefore, neuroanatomy lessons exploit all the options available ranging from basic functional anatomy maps, ultrasound of peripheral nerves to the opportunity to use DICOMs of clinical cases with a highly relevant neuroanatomical correlation. The same options are now being offered also to lessons devised for residents in neurology, neurosurgery, psychiarty and childhood neurology, to link easily neuroanatomy knowledge to the understanding of clinical cases both for diagnosis and patients' management. Furthermore, we are providing consultants with the opportunity to consolidate their knowledge in specific courses detailing, for example, the peripheral nervous system anatomy and how to approach it with the US. As the next step, we are implementing virtual tools in all teachings related to neuroanatomy at all levels (from the most basic to the most advanced), aiming also to detect the impact on the learning curve of all these implementations.

CONCLUSION

A digital and innovative approach to human anatomy teachings, and specifically neuroanatomy, can provide students with solid neuroanatomy knowledge to support clinical practice. Furthermore, empowering them by giving an active role in the learning process can make their path in becoming the next generation of health-care professionals smoother and more robust.

ACKNOWLEDGEMENTS

We thankfully acknowledge the *Anatomage Europe team* in supporting us during the on-line and on-site training activities of the *peer-to-peer* tutors.

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IJAE
ITALIAN JOURNAL OF
ANATOMY AND EMBRYOLOGY

Firenze University Press
www.fupress.com/ijae

OPEN ACCESS

Citation: Tambuzzi, S., Cattaneo, C., & Amadeo, A. (2024). A narrative review of astrocytes and suicide in psychiatric disorders. *Italian Journal of Anatomy and Embryology* 128(2): 135-146. https://doi.org/10.36253/ijae-15787

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

A narrative review of astrocytes and suicide in psychiatric disorders

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Abstract. Suicide is a real public health problem today, and in recent decades its possible neurobiological basis has been intensively studied. One particular strand of research has focused on suicide deaths and psychiatric disorders, with ample evidence for molecular mechanisms related to astrocytic abnormalities. The scope of the articles and their compilation over a period of many years has resulted in old, current and new knowledge being scattered across a large number of sources. The purpose of this narrative literature review is therefore to bring all this information together and summarize it in a single work that can be useful for those approaching this topic for the first time, for those looking for current evidence, and finally for those interested in exploring new frontiers of research. A comprehensive literature search has clearly shown that there are numerous converging findings indicating astrocyte changes in various biomarkers, particularly in the dorsolateral prefrontal cortex of suicidal individuals suffering from major depressive disorder. There is very little evidence for other brain regions and psychiatric disorders. Although these are preliminary results, they are encouraging and future studies could gradually overcome the limitations in the currently available literature and contribute to a better understanding of the etiopathological mechanisms of the occurrence of some of the main psychiatric pathologies leading to suicide.

Keywords: suicide, astrocytes, biomarkers, psychiatric disorders, major depressive disorder.

LIST OF ABBREVIATIONS

ACC: anterior cingulate cortex
ALDH1L1: aldehyde dehydrogenase 1 family member L1
AQP4: aquaporin 4
CA: cornus ammonis
Cb: cerebellum
CN: caudate nucleus
CNR2: cannabinoid receptor 2
CRYAB: crystallin alpha B
DCN: dorsal caudate nucleus

dlPFC: dorsolateral prefrontal cortex
dvPFC: dorsoventral prefrontal cortex
EEAT1: excitatory amino acid transporter 1
EEAT2: excitatory amino acid transporter 1
GFAP: glial fibrillary acidic protein
GJA1: gap junction protein alpha 1 (also known as connexin 43 CX43)
GJB6: gap junction protein beta 6 (also known as connexin 30 CX30)
GLUL: glutamine synthetase (also known as glutamate-ammonia ligase)
GPR55: G protein-coupled receptor 55
GRIK2: glutamate ionotropic receptor kainate type subunit 2
H3K9me3: Histone 3 lysine 9 trimetylaition
HIPP: hippocampus
ICAM1: Intercellular Adhesion Molecule 1
LC: locus coeruleus
MD: mediodorsal thalamus
MDD: major depressive disorder
NEBL: nebulette
OFC: orbitofrontal cortex
PVRL3: poliovirus receptor-like 3 protein (also known as Nectin 3)
PVC: primary visual cortex
PMC: premotor cortex
ROPN1B: rhophilin associated tail protein 1B
S100b: S100 calcium binding protein B
SCZ: schizoprenia
SOX9: SRY-box transcription factor 9
TrkB.T1: truncated isoform of the receptor tyrosine receptor kinase B
VIM: vimentin

1. INTRODUCTION

Among the prevalent public health and social impact problems worldwide, suicide plays an extremely important role as one of the leading causes of death, accounting for approximately 800,000 deaths per year (Claveria, 2022). Globally, suicides are most commonly observed in men and adults, with incidence rates varying between population groups in different countries. In this context, the World Health Organisation gives a standardised suicide rate per age of 5.5 deaths per 100,000 inhabitants for Italy (WHO, 2019). Considering the impact that the phenomenon of suicide has at different levels (health, epidemiology, public health and economy), suicide prevention is one of the main priorities in the health sector. The World Health Organization (WHO) has also recognized the importance of this phenomenon and has identified suicide as one of the most important research topics for investigating the causes and identifying effective prevention measures (Tambuzzi et al., 2024). However, this is a challenging task, as suicide is a phenomenon with a multifactorial aetiology that varies even from country to country, although the most important risk factors include psychiatric illness, alcohol abuse, family dysfunctionality and childhood trauma, socioeconomic status and a previous suicide attempt (Turecki and Brent, 2016). However, of all the risk factors for suicide, psychiatric disorders are the most important, and the population with such disorders has a 10 to 30 times higher risk than the general population (Song et al., 2020). The most common psychiatric diagnoses associated with suicide include depressive syndromes, schizophrenic spectrum disorders and personality disorders. Of all these diagnoses, major depressive disorders have an increasing impact on the overall burden of disease and account for 65% to 90% of suicides (Tambuzzi et al., 2024).

In this general and multifaceted context, a number of studies have accumulated in recent decades that have brought to light the presence of organic-structural brain alterations in mentally ill individuals and those who have died by suicide, highlighting abnormalities in glial populations (astrocytes, microglia and oligodendrocytes) as a possible etiopathological role. In particular, possible correlations exist in postmortem gene expression in the brain, morphological changes, cytokines and neurotrophic factors that indicate an inflammatory response or altered neuroplasticity (Liu et al., 2022; Yamamoto et al., 2024). Of all glial cells, astrocytes are the most numerous and have the greatest influence on brain function. Having long been considered merely accessory cells, it has been shown to date that they play a much more important and complex role, performing diverse and complex neuronal functions such as: neuronal metabolism (Choi et al., 2012; Hall et al., 2014; Marina et al., 2020); regulation of synaptic function and neuronal plasticity (interaction with neurotransmitters) (Arizono et al., 2020; Corkrum et al., 2020); synaptic remodelling during early brain development (Chung et al., 2016; Koeppen et al., 2018); protection of neurons from toxic substances (role in blood-brain barrier and immune defence) (Correa et al., 2011; Bell et al., 2011); and response to pathological injury through a process known as reactive astrogliosis (Liddelow and Barres, 2017; Escartin et al., 2021). Therefore, astrocytes are known as particularly dynamic cells that, due to their multiple functions, are able to continuously regulate the physiology of the brain to meet the demands of neuronal activity in a timely manner. They are also able to respond with functional and morphological changes to alterations in neurotransmitter systems, which play a role both in the pathophysiology of mood disorders (O'Leary and Mechawar, 2020) and in the therapeutic response to psychotropic and neuroprotective medications (Coyle and Schwarcz, 2000; Koyama, 2015). In particular, reduced expression of glutamate transporters has been found in people with depression, leading to a reduced ability of astrocytes to take up glutamate released by neurons at the synapse (Choudary et al., 2005; Bernard et al., 2011; Chandley et al., 2013; Medina et al., 2016). Thus, reduced astrocyte activity may contribute to dysfunction of the glutamatergic system, an essential mechanism for neuronal communication and synaptic plasticity (Miguel-Hidalgo et al., 2010; Parkin et al., 2018; Power and Sodhi, 2019). This may explain the correlation between altered astrocyte function and symptoms of major depression, a major cause of suicide (Rajkowska et al., 2013; Koyama, 2015). More in general, there has been evidence that astrocytic dysfunction can contribute also to the development of anxiety disorders and schizophrenia, which are other known risk factors for suicide (Rajkowska, 2000; Webster et al., 2001; Bowley et al., 2002; Webster et al., 2005; Altshuler et al., 2010; Kim et al., 2018). Over the years, however, it became apparent that a specific subpopulation of astrocytes expressing the glial fibrillary acidic protein (GFAP, the most important astrocyte marker) as a component of the intracellular intermediate filaments was altered (Lyck et al., 2008). In particular, Miguel-Hidalgo et al. were among the first to show that the density of immunoreactive (IR) GFAP astrocytes in the dorsolateral prefrontal cortex (dlPFC) of individuals suffering from major depression decreased in a positive correlation with age (Miguel-Hidalgo et al., 2000). In particular, in younger adults (30-45 years), the density of these astrocytes was below the minimum value of the range found in the healthy control group, in contrast to older depressed individuals (46-86 years). Si et al. further investigated these age-related changes in the dorsolateral prefrontal cortex and showed that the density of GFAP-IR atrocytes was significantly lower in depressed subjects under 60 years of age than in the healthy control population (Si et al., 2004). In addition, the density of GFAP-IR astrocytes was found to correlate with age at onset of depression. From this, the authors concluded that reduced GFAP levels may contribute to the pathophysiology of depression, particularly in relatively young people.

Precisely because of the importance of astrocytes in the overall function of the nervous system and their etiologic link to psychiatric disorders and suicide, research has focused mainly on them, with the result that old, current and new knowledge have accumulated and are scattered over many sources. Therefore, the aim of this narrative literature review is to summarize all this information specifically related to astrocytes in suicidal patients with psychiatric disorders in a single work that can be useful for those approaching this aspect for the first time, for those in search of insights, and finally for those interested in exploring new frontiers of research to find innovative approaches to prevent and protect the health of individual patients and the community as a whole.

2. MATERIAL AND METHODS

A literature search was conducted in the most common electronic databases (PubMed, Scopus, Medline, Google Scholar and Web of Science) up to October 15, 2024, in which the following combinations of text protocols "astrocytes" were combined individually and randomly with the Boolean operator "and": "suicide", "biomarker", "psychiatric disorder". Based on PubMed, the search for "astrocytes and suicide" yielded 125 articles. The combination "astrocytes and suicide and psychiatric disorder" yielded 50 articles. The addition of "biomarkers" reduced the number of articles. Of the total number, all articles dealing with the analysis of astrocytes in the postmortem brain of individuals who died by suicide and suffered from a psychiatric disorder were included. Therefore, all studies dealing only with suicide cases without psychiatric disorder were not the main focus of this review. All studies conducted on animals were excluded. The remaining databases were also reviewed, with duplicates removed. Overall, only English-language full-text articles were considered, with dated publications also included. Finally, all bibliographies of the selected articles were checked for further relevant articles.

3. RESULTS

The most important changes in the astrocyte population in the postmortem brain of suicidal patients with psychiatric disorders described in the literature so far are a lower astrocyte density and number, morphological alterations and changes in protein expression compared to control groups of healthy individuals. The majority of post-mortem studies on this topic had shown that in several regions of the prefrontal cortex of brains of individuals with depression and died by suicide, the density and number of different types of glial cells, especially astrocytes, had decreased (Yamamoto et al., 2024). In fact, Miguel-Hidalgo et al. reported a reduction in glial packing density in the orbitofrontal cortex (OFC) in alcohol dependence (Miguel-Hidalgo et al., 2006). However, at the same time, Hercher et al. observed an increase of glial density in the anterior cingulate cortex (ACC) of suicide completers with alcohol dependence (Hercher et al., 2009). Furthermore, Torres-Platas et al. reported that cortical astrocytes positive for Golgi staining had significantly larger cell bodies and a greater number of nodes, an average number of branches, a total length of branches and a total number of spines, and more branching processes in the anterior cingulate cortex (ACC) of depressed suicides, suggesting a neuroinflammatory theory of depression and suicide (Torres-Platas et al., 2011). Finally, Hercher et al. pointed out that the spatial distribution of astrocytes in suicide victims with schizophrenia and bipolar disorder differed from that of the control group (Hercher et al., 2014).

3.1. Alterations in GFAP-IR astrocytes in suicide deceased and psychiatric disease

The psychiatric condition that has been most studied in this context is major depressive disorder (MDD), since 2013, when Chandley et al. showed in a post-mortem study of the locus coeruleus (LC) of MDD patients that most decedents who died by suicide had a significantly lower density of GAFP-IR astrocytes than corresponding control subjects (Chandley et al., 2013). Later, Nagy et al. demonstrated that GFAP and its transcription were reduced in the dorsolateral prefrontal cortex (dlPFC; Nagy et al., 2015) of depressed suicidal patients (Table 1). Interestengly, Cobb et al. showed that there were a decrease in the density of GFAP-IR astrocytes in the hippocampus (HIPP) between patients with and without depressive suicide, and their density in certain areas (cornus ammonis – CA 2/3) was inversely correlated with the duration of depression and treatment in suicide victims (Cobb et al., 2016). Remarkably, it was significantly reduced only in depressed individuals not taking antidepressant medication, while this was not the case in depressed individuals receiving specific therapy (Table 1). Almost simultaneously, Torres-Platas et al. described a decrease in GFAP and its transcription in mediodorsal thalamus (MD) and caudate nucleus (CN) in the brains of depressed and suicide individuals and similar levels to healthy controls in primary visual cortex (PVC), premotor cortex (PMC) and cerebellum (Cb) (Torres-Platas et al., 2016; Table 1). Rajkowska et al. examined GFAP-IR astrocytes in the white matter adjacent to the dorsoventral prefrontal cortex (dvPFC) in individuals who suffered from depression and some of them died by suicide (Rajkowska et al., 2018). In these subjects, a reduction in the density and tissue coverage of GFAP-IR astrocytes was found, while the average size of the astrocytic bodies remained unchanged (Table 1). Even more recently, O'Leary et al. demonstrated by immunohistochemistry (IHC) that the density of GFAP-IR astrocyte subpopulations in the brain (dorsal caudate nucleus – DCN, dorsolateral prefrontal cortex – dlPFC, and mediodorsal thalamus – MD) of depressed individuals who died by suicide was statistically reduced compared to control groups of healthy individuals (O'Leary et al. 2021; Table 1).

With regard to other psychiatric disorders, changes in GFAP-IR atrocytes have been studied almost exclusively in postmortem brain tissue from individuals who did not die by suicide. For the purposes of this literature review, therefore, only the study by Zhang et al. must be considered, in which schizophrenia (SCZ) was assessed (Zhang et al., 2020). No changes in the mRNA concentration of GFAP in the dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) between suicide and non-suicide patients with SCZ and their matched controls were reported (Table 1).

3.2. Alterations in other astrocytic markers in suicide deceased and psychiatric disease

Since around 2010, another front of astrocyte research has been pursued with the aim of investigating regional variations in the distribution and morphology of astrocytes in the brains of suicidal and psychiatrically ill individuals. In fact, the GFAP protein identifies only part of the astrocytes and may only incompletely represent the astrocytic phenotype (O'Leary and Mechawar, 2020; Jurga at al., 2021). For this reason, other protein markers have also begun to be characterised.

The first to be studied in the postmortem brain of subjects with depression and suicide were glutamine synthetase (also known as glutamate-ammonia ligase, GLUL) and S100b (a calcium binding protein), whose mRNAs were examined by Klempan et al. only in the dorsolateral prefrontal cortex (dlPFC), with inconclusive results, as both not-statistically-significant increases and decreases were found (Klempan et al., 2009; Table 2).

The study of GLUL was later also taken up in two other articles (Nagy et al., 2015; Zhang et al., 2020). Nagy et al. examined the dorsolateral prefrontal cortex (dlPFC) in suicidal and depressed individuals and found a decrease in GLUL expression. On the other hand, Zhang et al. focused on the dorsolateral prefrontal cortex (dlPFC) of suicidal and schizophrenic individuals and reported a decrease in GLUL expression, that was not present in the anterior cingulate cortex (ACC) (very similar val-

Table 1. Astrocytic GFAP changes in suicide victims in relation to the psychiatric disorder they suffered from.

Psychiatric disorder	Reference (year)	Astrocyte marker	Type	Brain areas										
				LC	dlPFC	HIPP	MD	CN	PVC	PMC	Cb	dvPFC	DCN	ACC
MDD	Chandley et al. (2013)	GFAP	Protein	↓										
			mRNA	↓										
	Nagy et al. (2015)	GFAP	mRNA		↓									
	Cobb et. al. (2016)	GFAP	Protein			↓								
	Torres-Platas et al. (2016)	GFAP	Protein				↓	↓	»	»	»			
			mRNA				↓	↓	»	»	»			
	Rajkowska et al. (2018)	GFAP	Protein									↓		
			mRNA									↓		
	O'Leary et al. (2021)	GFAP	IHC		↓		↓						↓	
SCZ	Zhang et al. (2020)	GFAP	mRNA		»									»

ues compared to the control cases). The study of glutamate metabolism in relation to depression and suicide was further investigated by Zhao et al. (2016). The dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) were examined in young MDD patients who died by suicide, MDD patients who died from non-suicidal causes, and comparable control subjects. Components of the glutamate-glutamine cycle with astrocytic localization (GLUL and excitary aminoacid transporters, EAAT1 and EAAT2) were found to be decreased in the dorsolateral prefrontal cortex (dlPFC) of suicidal and depressed patients. EEAT1 (excitatory amino acid transporter 1) and EEAT2 (excitatory amino acid transporter 2) were further specifically studied by Chandley et al. in the locus coeruleus (LC) of suicidal and depressed subjects, showing a significant decrease (Chandley et al., 2013). A similar decrease in EEAT1 only was also demonstrated in the dorsolateral prefrontal cortex (dlPFC) by Nagy et al. (Nagy et al., 2015; Table 2).

On the other hand, S100b was examined in more detail by Zhang et al. in the brains of suicidal and depressed people with psychotic symptoms, who showed similar values to healthy controls in both dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) (Zhang et al., 2021; Table 2).

In chronological order, the new astrocytic marker studied was ICAM1 (Intercellular Adhesion Molecule 1) by Miguel-Hidalgo et al. which showed a decrease in orbitofrontal cortex (OFC) in suicidal and depressed individuals compared to control subjects (Miguel-Hidalgo et al., 2011; Table 2). Since then, no further studies have been conducted on this specific marker.

Subsequently, the GJA1 protein (gap junction protein alpha 1), also known connexin 43 (CX43), was examined in the orbitofrontal cortex (OFC) of the brain of suicidal individuals with depression and alcoholism, and no differences were found compared to healthy controls (Miguel-Hidalgo et al., 2014). The study of the GJA1 protein was not further developed in subsequent years; instead, mRNA analysis of this marker was preferred. Indeed, the mRNA levels of GJA1 were examined by Nagy et al. in the dorsolateral prefrontal cortex (dlPFC) of suicidal and depressed individuals, as well as GJB6 (gap junction protein beta 6, also known as connexin 30 – CX30), ALDH1L1 (aldehyde dehydrogenase 1 family member L1), and SOX9 (SRY-box transcription factor 9) (Nagy et al., 2015). A statistically significant decrease was demonstrated for all these markers compared to healthy controls (Table 2). GJA1 and GJB6 were further investigated by Nagy et al. in 2017, again in suicidal and depressed subjects, and showed a significant decrease in mRNA levels compared to controls in mediodorsal thalamus (MD), premotor cortex (PMC), primary visual cortex (PVC), and caudate nucleus (CN) (Nagy et al., 2017). In the cerebellum (Cb), however, a decrease in GJA1 was observed, while GJB6 increased. In 2019, Tanti et al. also examined GJB6 in suicidal and depressed subjects and demonstrated a significant decrease in mRNA levels compared to controls in the anterior cingulate cortex (ACC) (Tanti et al., 2019; Table 2).

More recently, the study of ALDH1L1 was also resumed in two articles on the brains of suicide people with schizophrenia (Zhang et al., 2020) and with depression (Zhang et al., 2021. It was found that ALDH1L1 was decreased in the dorsolateral prefrontal cortex (dlPFC) of suicidal and schizophrenic subjects, while there were no statistical differences in the dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) of suicidal and depressed subjects compared to controls (Table 2).

The most recent area of research was conducted in 2021, when O'Leary et al. characterised another astro-

Table 2. Astrocytic changes in suicide victims in relation to the psychiatric disorder they suffered from.

Psychiatric disorder	Reference (year)	Astrocyte marker	Type	Brain areas								
				dlPFC	ACC	LC	OFC	MD	PMC	PVC	CN	Cb
MDD	Klempan et al. (2009)	GLUL	mRNA	»								
MDD	Nagy et al. (2015)	GLUL	mRNA	↓								
SCZ	Zhang et al. (2020)	GLUL	mRNA	↓	»							
MDD	Zhao et al. (2016)	GLUL	Protein	↓	»							
MDD	Chandley et al. (2013)	EEAT1	mRNA			↓						
		EEAT2	mRNA			↓						
MDD	Nagy et al. (2015)	EEAT1	mRNA	↓								
MDD	Zhao et al. (2016)	EEAT1	mRNA	↓	»							
		EEAT2	mRNA	↓	»							
MDD	Klempan et al. (2009)	S100b	mRNA	»								
MDD and psycothic symptons	Zhang et al. (2021)	S100b	mRNA	»	»							
MDD	Miguel-Hidalgo et al. (2011)	ICAM1	IHC				↓					
MDD and alcoholism	Miguel-Hidalgo et al. (2014)	GJA1 (CX43)	Protein				»					
MDD	Nagy et al. (2015)	GJA1 (CX43)	mRNA	↓								
MDD	Nagy et al. (2017)	GJA1 (CX43)	mRNA					↓	↓	↓	↓	↓
MDD	Nagy et al. (2015)	GJB6 (CX30)	mRNA	↓								
MDD	Nagy et al. (2017)	GJB6 (CX30)	mRNA					↓	↓	↓	↓	
MDD	Tanti et al. (2019)	GJB6 (CX30)	mRNA		↓							
MDD	Nagy et al. (2015)	SOX9	mRNA	↓								
MDD	Nagy et al. (2015)	ALDH1L1	mRNA	↓								
SCZ	Zhang et al. (2020)	ALDH1L1	mRNA	↓								
MDD	Zhang et al. (2021)	ALDH1L1	mRNA		»							
MDD	O'Leary et al. (2021)	VIM	IHC	↓				↓			↓	

cytic population using a new marker identified in vimentin (VIM), also a type III intermediate filament like GFAP (O'Leary et al., 2021). It had the additional advantage that it also highlights blood vessels and thus facilitated the identification of astrocytes. After an initial evaluation in healthy human brain tissue (O'Leary et al., 2020), this marker was used to further characterise astrocyte subpopulations in suicidal and depressed patients. In particular, it was demonstrated a statistically significant decrease of VIM-IR astrocytes in the dorsolateral prefrontal cortex (dlPFC), mediodorsal thalamus (MD), and caudate nucleus (CN) compared to healthy controls (Table 2). In view of the close connection between astrocytes and blood vessels, the density of the blood vessels was also examined in the same regions using the CD-31 marker. It was found that similar to GFAP-IR astrocytes, the density of VIM-IR astrocytes was also reduced in the brains of depressed suicide victims compared to control subjects. In contrast, the density of CD-31-positive blood vessels was similar between the two groups, except in the prefrontal white region, where vascularization was increased.

To date, astrocyte markers such as cannabinoid receptor 2 (CNR2; Garcia-Gutierrez et al., 2018), G protein-coupled receptor 55 (GPR55; Garcia-Gutierrez et al., 2018), crystallin alpha B (CRYAB; Ernst et al., 2011), truncated isoform of the receptor tyrosine receptor kinase B (TrkB.T1; Ernst et al., 2009), and aquaporin 4 (AQP4; Bernard et al., 2011; Rajkowska et al., 2013; Medina et al., 2016; Rosu et al., 2019; Genel et al., 2021) have not yet been studied in suicides of individuals who were affected by psychiatric disorders, but only in one of the two scenarios: patients with a psychiatric disorder not died by suicide or patients died by suicide but not affected by any psychiatric disorders. Regarding AQP4 specifically, Medina et al. analysed a postmortem study group of 13 individuals affected by MDD, but only six of whom had died by suicide. Overall, a decrease in AQP4 mRNA expression was found in the hippocampus of the study subjects compared to the control group, but it is not possible to distinguish between those who died by suicide and those who did not (Medina et al., 2016).

Finally, for the sake of completeness, only one study is reported in which DNA methylation in the brains of suicidal and depressed individuals was investigated. The study conducted by Nagy et al. revealed a decrease in the methylation of the GRIK2 (glutamate ionotropic receptor kainate type subunit 2) and NEBL (nebulette) genes and an increase in the PVRL3 (poliovirus receptor-like 3 protein), also known as Nectin 3, and ROPN1B (rhophilin associated tail protein 1B) genes in the dorsolateral prefrontal cortex (dlPFC) compared to healthy subjects (Nagy et al., 2015).

There is also a single study in the literature on epigenetic silencing that shows an increased rate of silencing of the Histone 3 lysine 9 trimetylaition (H3K9me3), again in the dorsolateral prefrontal cortex (dlPFC) of suicidal and depressed individuals (Nagy et al., 2017).

4. DISCUSSIONS

In recent decades, much research has been conducted on suicide in general, and the study of the brains of suicide individuals has become increasingly important in determining possible organic causes of suicidal behaviour. In particular, the study of the postmortem neuroanatomy of the brains of individuals who have died by suicide continues to be of great interest due to the potential scientific implications that may arise (Yamamoto et al., 2024). Indeed, glial cells, particularly astrocytes, have been reported to be involved in suicide, although the pathological mechanism of glial activity and the risk of suicidal behaviour remain unclear. In this general context, another research focus has gradually been placed on the study of the neuroanatomy of the brain cells of people who died by suicide but were also affected by psychiatric disorders. The intensive research activity on this topic has led to many results being scattered in various publications. It was therefore considered useful to compile a review of the literature focusing specifically on the astrocytic changes described and reported to date.

It is clear, and on this point the literature agrees, that astrocyte populations in the brains of individuals suffering from psychiatric disorders who have died by suicide are characterised by changes in their density and number, with alterations in protein expression compared to control groups of healthy individuals. This has been particularly observed in major depression, a psychiatric disorder that has been by far the best studied to date (O'Leary and Mechawar, 2020). However, the overall picture became particularly complicated as research tried to investigate specific astrocyte subpopulations using specific markers in specific brain regions in different psychiatric pathologies. The results have been sometimes contradictory, but this is not surprising since, as clearly stated in the literature, any psychiatric disorder can be considered a confounding factor (Qi et al., 2019). It follows that although all studies that have contributed to this topic have been designed as case-control studies comparing suicide cases with psychiatric disorders with healthy control cases, each study person (i.e. with a psychiatric disorder) is an absolutely unique individual. In general, each person is difficult to categorise, and this is even more so when the main selection criteria concern psychiatric disorders and the suicide event. As mentioned above, suicide is a multifactorial event involving several aspects, and it is not yet known what influence neuroanatomy has on the ability to commit suicide, also because there is often a lack of appropriate case controls (Yamamoto et al., 2024). Furthermore, in all studies conducted, the degree of internal suffering of suicidal individuals was unknown, as was often the motivation. In most cases, information on the psychiatric illnesses from which the test subjects suffered was also missing. With a few exceptions, the duration and severity of the psychiatric illness were not known and, above all, any treatment the person was undergoing was not taken into account. And it is precisely here that the greatest limitation of all previous work in the literature on this topic (but also on suicide in general) becomes apparent, namely the little or no consideration of the role of the interaction of the individual's biology with the environment. Indeed, the literature shows that even the environment in the broadest sense, i.e. external exposure to air and water, the consumption of medicatons and stimulants (e.g. smoking, alcohol and illicit drugs) appears to make a non-negligible direct contribution to suicide (Tambuzzi et al., 2024). In addition, numerous environmental pollutants are considered neurotoxic due to their effects on brain cell plasticity and can cause the onset of psychiatric pathologies, which also indirectly contributes to suicide (Costa et al., 2017; Calderón-Garcidueñas et al., 2008; Jones and Miller, 2008; Munzel and Daiber, 2018). At the same time, it is obvious how difficult it can be to take all these variables into account in a single study.

Overall, it is very interesting, precisely because of the great inter-individual biological variability that characterises the suicide victims with a psychiatric disorder selected in the individual studies, that converging tendencies nevertheless emerge in this panorama. In addition to the general observations of reduced overall astrocyte density, significant results were obtained in the search for specific astrocyte markers, in particular GFAP. Over a period of about 20 years, almost all publications found that the population of GFAP-IR astrocytes was statistically reduced in the brains of individuals who had died by suicide and suffered from depression compared to healthy controls (Chandley et al., 2013; Nagy et al., 2015; Cobb et al., 2016; Torres-Platas et al., 2016; Rajkowska et al., 2018; O'Leary and Mechawar, 2020). This was demonstrated in several brain regions, but particularly in the dorsolateral prefrontal cortex (dlPFC) cortex, which was by far the most studied. This was also recently confirmed by O'Leary et al., not only in the dlPFC but also in the dorsal caudate nucleus (DCN) and mediodorsal thalamus (MD), confirming earlier observations that brain involvement is more extensive than it initially appeared (O'Leary et al., 2021). However, postmortem brain tissue from individuals with schizophrenia and bipolar disorder has also been shown to be affected, with a reduced number and density of GFAP-IR astrocytes, giving a picture of diffuse astrocytopathy (Webster et al., 2001; Altshuler et al., 2010). However, studies specifically focusing on suicide victims affected by psychiatric diseases other than depression are still lacking.

From all this it can be concluded that the presence of neuroanatomical changes in the number and density of GFAP-IR astrocytes in certain brain regions of persons with psychiatric disorder who have died by suicide has so far been documented quite solidly in the literature.

Equally interesting is the fact that the studies currently published in the literature seem to indicate that further changes in astrocyte biomarkers may occur in the brains of these individuals. In this overall picture, the most promising research fronts appear to lie in the analysis of the GLUL protein (Klempan et al. 2009; Nagy et al., 2015; Zhao et al., 2016; Zhang et al., 2020), ALDH1L1 (Nagy et al., 2015; Zhang et al., 2020, Zhang et al., 2021) and VIM (O'Leary et al., 2020; O'Leary et al., 2021). These are endocellular or membrane proteins that are directly involved in astrocyte metabolism in various ways and for which several studies have shown a statistically significant reduction in certain brain regions (again, mainly in the dorsolateral prefrontal cortex - dlPFC) of individuals who died by suicide and suffered from depression or in some cases even schizophrenia compared to healthy controls.

It is also of great importance that there are more recent confirmations for the above-mentioned proteins from studies after 2020, which prove the scientific significance of these results. As far as the other markers examined are concerned, the current results are still preliminary and in some cases cannot be clearly interpreted. Some of them, such as S100b and AQP4, have been studied more in the context of depression or suicide, but not yet sufficiently in situations where both variables coexist, i.e. suicide and psychiatric pathology. To date, there is also very limited evidence for studies of DNA methylation and gene silencing of astrocyte populations in the brains of suicidal and psychiatrically ill individuals.

Overall, it is clear that this is a ground-breaking field of research with very significant preliminary results. However, it also highlights the many limitations that currently exist. In particular, previous studies have only analysed European populations. In addition, brain regions have been discussed, such as the right or left hemisphere and white or grey matter, where astrocyte concentrations are known to differ (Pelvig et al., 2008; Verkhratsky et al., 2017; Forrest et al., 2023). Furthermore, the sample sizes were small, and the different methods and outcome measures used in the different studies may affect the reliability and accuracy of the results. In addition, as previously mentioned, the effects of medications and other medical therapies used in patients with psychiatric disorders on brain biology should be further discussed. Future research on this topic should take these aspects into account, and in particular it would be interesting to try to relate the data on drug therapy and exposure to possible toxic substances to those from neuroanatomical examination of the brain. The close relationship between astrocytes and the blood vessels of the brain, which form the blood-brain barrier, is well known (Kealy at al., 2020). It is therefore quite conceivable that when astrocyte density is reduced, as has already been described in the literature, defences against toxic substances of various kinds are less effective and brain cells, including the astrocytes themselves, are exposed to chronic stress, possibly leading to pathological changes and psychiatric disorders. Other variables not yet considered that should be investigated are the age and sex of the victims, the assessment of the post-mortem interval and whether the patient died during the day or at night, which could affect the markers of glial activation after death (Wruck and Adjaye, 2020; Cabrera-Mendoza et al., 2020; Yu et al., 2023).

Apart from these considerations, the fact that the researches have been conducted on postmortem human brain tissue is certainly a strength, as preclinical (animal) studies are of limited use in this particular area of research. Moreover, such an approach makes it possible to emphasise that, although often forgotten, much can be learned from the dead to contribute to the well-being of the living. And pursuing the goal of a better interpretation of suicide risk through a more careful assessment of suicide risk stratification, especially in special populations, is precisely one of these goals. Although these are still preliminary results, it is important that this line of research continues, as it could progressively contribute to a better understanding of the etiopathological mechanisms of the occurrence of some of the main psychiatric pathologies that lead to suicide. Moreover, it is possible that, based on this new knowledge, innovative therapeutic perspectives can be implemented, as well as new approaches to prevent and protect the health of individual patients and the community as a whole.

ACKNOWLEDGMENTS

The authors thank the PhD course in Environmental Sciences at the University of Milan for the opportunity to carry out this research.

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Finito di stampare da
Logo s.r.l. – Borgoricco (PD) – Italia

I J A E
Italian Journal of Anatomy and Embryology
Vol. 128, N. 2 – 2024

€ 26,00 (for Italy)